MICROBIOTEST PROTOCOL

ASSESSMENT OF FUNGICIDAL EFFICACY OF COPPER OXIDE IMPREGNATED FABRIC AFTER TWELVE HOURS OF EXPOSURE

Trichophyton mentagrophytes

Prepared for:
Cupron Inc.
Suite 123
800 East Leigh Street
Richmond, VA 23219

Testing Facility:
MICROBIOTEST
A Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

February 26, 2013

OBJECTIVE AND TEST OVERVIEW:

This test is designed to evaluate fungicidal effectiveness of a fabric material impregnated with an antimicrobial ingredient (copper oxide). A treated test fabric and an untreated control fabric will be subject to the same level of fungal challenge; held for a contact time; and recovered for survived fungi. The fungal load from the test fabric will be compared to that from the control fabric to determine fungicidal efficacy of the test fabric.

A fabric representative of "light" fabrics such as clothing, bed linens, kitchen linens, bath linens, etc. will be tested. The control fabric will be of the same fiber type and fabric construction as the test sample but containing no antimicrobial finish.

The test fabric may be marketed as reusable or disposable. This protocol will evaluate the test fabric for fungicidal efficacy both "as is" and after simulated wash and drying cycles, which will include simulated environmental stressing to demonstrate the efficacy of the product over prolonged use If the test fabric is effective for reusable, it shall be good for disposable.

The fungal challenge test design is based on the AATCC[1] Test Method AATCC 100-2004. The method follows the principles stipulated in the U.S. Environmental Protection Agency (EPA) guidelines Pesticide Assessment Guidelines Subdivision G, Series 91-52 (a) (1) (i) and (b) (1), Pesticide Assessment Guidelines Subdivision G, Series 91B, 91-51, and EPA DIS/TSS-14 and EPA DIS/TSS-16[2].

TESTING CONDITIONS:

Three lots of one type of test fabric will be evaluated along with one lot of an untreated control fabric will be treated in parallel and will be designated as one "set".

One set of fabric, designated as the 0 wash condition will be tested as is (i.e., without pre-stressing or washing). The second set, designated as the 20 x wash/drying condition will be tested after 20 wash/drying cycles, each of which will include simulated environmental stressing.

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[1] AATCC: American Association of Textile Chemists and Colorists
[2] DIS/TSS: Disinfectant Technical Science Section

Each condition will include a fungicidal efficacy evaluation using five replicate fabric carriers for each of the three lots of the test fabric and the untreated control fabric (single lot) at one contact time (twelve hours) using *Trichophyton mentagrophytes* as the challenge microorganism with the aim of showing a three-log (99.9%) reduction of the test fabric over the control fabric in twelve hours.

Table 1 outlines the general procedures to be used for the two conditions (0 wash and 20 x wash/drying).

For the 0 wash condition, the test and control fabric will be evaluated for fungicidal efficacy under ideal conditions (no exposure to wash/drying or simulated environmental stressing).

For the 20 x wash/drying condition, the test and control fabrics will be exposed to a regimen to simulate consumer use conditions. The regimen will mimic in use conditions via simulated environmental stressing. The procedures will include exposure to high humidity (85-100% relative humidity (RH)) under incubation (36±2°C) followed by exposure to ultraviolet (UV) irradiation. In addition the fabric carriers will be inoculated with low levels of fungi to mimic recontamination of fabric during its life of use before exposure to a specified wash and drying procedure. These procedures will provide a worse-case scenario for the test and control fabric prior to use for the fungicidal efficacy evaluation.

A high-level fungal inoculum preparation will be used for the efficacy tests for both conditions (0 wash and 20 x wash/drying) whereas a low-level fungal inoculum preparation will be used for the 20 x wash/drying simulated use procedures.

All fungal inoculum preparations (high-level and low-level) will be suspended in synthetic sweat to mimic a simulated use biological challenge.

For the efficacy evaluations, the fabric carriers will be inoculated with a high-level inoculum preparation and incubated at 36±2°C under humid conditions (85-100% relative humidity (RH)) for the duration of the twelve hour contact time. At the conclusion of the contact time, each fabric carrier will be transferred to neutralizer and processed using stomaching procedures to extract any remaining survivors. Samples of the neutralizer recovery broth will be cultured and after appropriate incubation enumerated.

Table 1: General procedures to be used for the 0 wash and 20 x wash/drying conditions.	
Primary Samples*	**Summary**
Set 1 – Unwashed and Unstressed (0 wash)	Test as is – no pre-conditioning or stressing: 1. Fabric carriers will be exposed to UV irradiation at room temperature (20-25°C) in a biological safety cabinet for 30 minutes per side followed by air drying at room temperature (20-25°C) for at least 30 minutes before proceeding to the fungicidal efficacy test. 2. Twelve hour fungicidal efficacy evaluation performed. 3. Fungal challenge: Fabric carriers will be inoculated using an inoculum containing synthetic sweat as organic soil with a final concentration of $10^5 – 10^7$ conidia/mL (high-level preparation). 4. The inoculated fabric carriers will be incubated as soon as possible post-inoculation at 36±2°C, 85-100% RH until the conclusion of a twelve hour contact time in open Petri dishes. The contact time will be initiated immediately after inoculation. 5. At the conclusion of the twelve hour contact time, the fabric carriers will be neutralized and cultured.
Set 2 – Wash/Drying (20 cycles, including environmental stressing)	20 wash/drying cycles (to include environmental stressing and wash and drying conditions) : 1. Pre-conditioning (applicable prior to the first wash/drying cycle only): Fabric carriers will be incubated at 36±2°C, 85-100% RH for 24 hours followed by UV irradiation at room temperature (20-25°C) in a biological safety cabinet for one hour before the initial fungal challenge as outlined in Step 2, part b below. 2. Environmental stressing conditions: a. Fabric carriers will be dried at 36±2°C, 85-100% RH for two hours and UV irradiation at room temperature (20-25°C) in a biological safety cabinet for 15 min to mimic wear and tear (applicable to wash/drying cycles 2 – 20 only). b. Fungal challenge: Fabric carriers will be inoculated using and inoculum containing synthetic sweat as organic soil with a final concentration of $10^2 – 10^3$ conidia/mL (low-level preparation). c. Fabric carriers will be dried at room temperature (20-25°C) for 20-30 minutes. 3. Wash condition: Fabric carriers will be exposed to 80±15°C water containing a commonly used detergent, such as Woolite® and bleach for 10-12 minutes. 4. Rinse condition: Fabric carriers will be thoroughly rinsed with sterile tap water to assure that there is no residual detergent or bleach remaining that may kill the test microorganism or interfere with the ionic release or interfere with the fungal assay itself. 5. Drying condition: Fabric carriers will be tumble dried at 175±30°F (62-96°C) for 20 - 30 minutes. 6. Repeat steps 2 – 5 until the completion of 20 cycles.

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Set 2 (continued) – Wash/Drying (20 cycles, including environmental stressing)	Test post 20 wash/drying cycles: 1. Fabric carriers will be exposed to UV irradiation at room temperature (20-25°C) in a biological safety cabinet for 30 minutes per side followed by air drying at room temperature (20-25°C) for at least 30 minutes before proceeding to the sanitizer test. 2. Twelve hour fungicidal efficacy evaluation performed. 3. Fungal challenge: Fabric carriers will be inoculated using an inoculum containing synthetic sweat as organic soil with a final concentration of $10^5 - 10^7$ conidia/mL. 4. The inoculated fabric carriers will be incubated as soon as possible post-inoculation at 36±2°C, 85-100% RH until the conclusion of a twelve hour contact time in open Petri dishes. The contact time will be initiated immediately after inoculation. 5. At the conclusion of the twelve hour contact time, the fabric carriers will be neutralized and cultured.
Others Notes	No. lots/replicates: 3 lots, 5 reps (test fabric) and 1 lot, 5 reps (control fabric)
	Target log reduction: 3-logs within 12 hours

Each set = 3 lots Test fabric + 1 lot Untreated control fabric

MATERIALS:

A. Test and control materials will be supplied by the sponsor of the study. The test materials will be tested as supplied by the sponsor unless directed otherwise. All applicable operations performed on the materials such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures the testing facility management that the materials have been appropriately tested for identity, strength, purity, stability, and uniformity as applicable. MICROBIOTEST will retain all unused test materials for a period of at least three months after completion of the test, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MICROBIOTEST, including, but not limited to:

1. Challenge microorganism (requested by the sponsor of the study): *Trichophyton mentagrophytes*, ATCC 9533

2. Miscellaneous laboratory equipment and supplies including microporous filtration apparatus, and laundrometer.

3. Media and reagents:
   a. Sterile tap water
   b. Woolite®, Reckitt Benckiser
   c. Bleach, commercially available
   d. Sterile saline solution (SS)
   e. Neutralizer: 2X Letheen Broth
   f. Sterile Phosphate Buffer Dilution Water (PBDW)
   g. Neopeptone Glucose Agar (NGA)
   g. Synthetic sweat (acidic)[3]
      - 0.5 g of L-histidine hydrochloride monohydrate
      - 5g of NaCl
      - 2.2g of $Na_2HPO_4 * 12H_20$
      - Dissolved in pure (deionized) water (985 mL)
      - Mixed with 15 mL of NaOH 0.1 M
      - Adjust pH to 5.5
      - Sterile filtered using 0.22µm filter

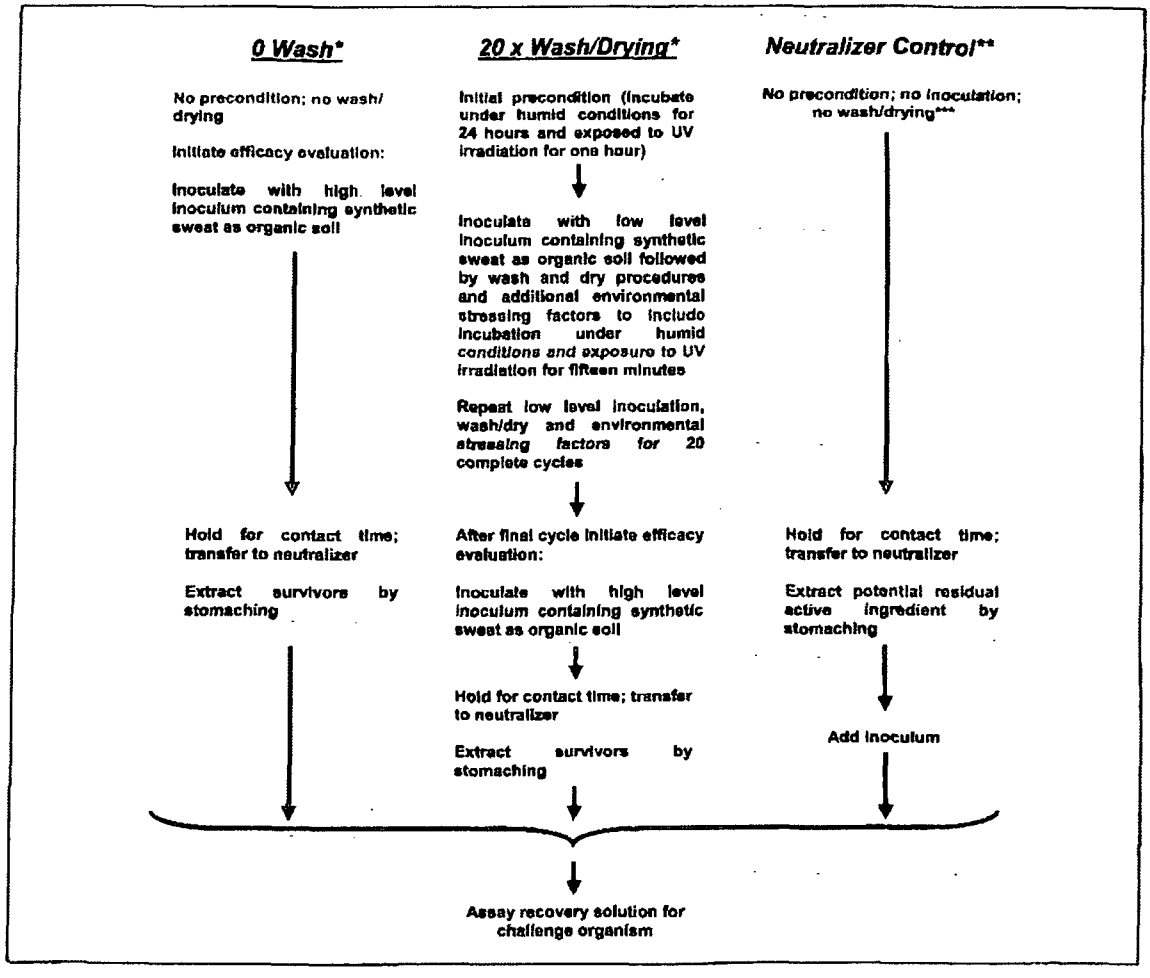
TEST SYSTEM IDENTIFICATION:

All test and control tube racks will be labeled with microorganism, test material identification (if applicable) and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation.

[3] Nakashima H, Miyano N, Takatuka T. Elution of metals with Artificial Sweat/Saliva from Inorganic Antimicrobials/Processed Cloths and Evaluation of Antimicrobial Activity of Cloths. *Journal of Health Science* 54 (4) 390-399 (2008).

EXPERIMENTAL DESIGN:

Figure 1: Study flow diagram depicting the general procedures.



Neutralizer: Neutralizer Effectiveness control

** Three lots of the test fabric and one lot of the untreated control fabric (five replicates each) will be tested.*
***Three lots of the test fabric only (single replicates each) will be tested.*
****Represents the worst-case scenario for this control.*

Note 1: For the 20 x Wash/Drying procedures, the contaminated test fabric will be air dried for 20-30 minutes before being subjected to the wash subsequent cycle procedures.
Note 2: Additional controls will be tested as detailed in Section D.

A. Inoculum preparation:

The fungus will be inoculated from the stock culture onto NGA plates and incubated at 25-30°C for ≥10 days, but ≤ 15 days or until sporulation occurs. When the cultures appear to be mature, the mycelial mats will be removed from the surface of at least five plates and macerated with SS in a sterile glass tissue grinder. The suspension will be filtered through sterile glass wool to remove the hyphae. The density of the conidial suspension will be determined by serially diluting the prepared culture in SS. Aliquots from selected dilutions will be plated on duplicate NGA plates. The plates will be incubated for 3-5 days at 25-30°C. The suspension will be stored at 2-10°C for up to four weeks for use in the test.

On the day of each use, the suspension will be adjusted to yield the desired inoculation range using Synthetic Sweat to yield the desired inoculation concentration range; this dilution will be documented and reported. The minimum dilution of inoculum to synthetic sweat used will be at least 1:10. This step will yield a fungal preparation with synthetic sweat as the carrier.

This process will be repeated as necessary for inoculation aspects as necessary.

B. Test and Control Material Preparation:

The treated test and untreated control fabric samples will be aseptically cut into 1 x 1 inch carriers. The fabric carriers may be tagged with clothing barbs for identification and tracking purposes.

The fabric carriers required for the 0 wash condition (and associated controls) will be exposed to UV irradiation at room temperature (20-25°C) in a biological safety cabinet for 30 minutes per side followed by air drying at room temperature (20-25°C) for at least 30 minutes. The fabric carriers will then be transferred to sterile Petri dishes before proceeding to the Fungicidal Efficacy Test (Section E).

The fabric carriers required for the 20 x wash/drying condition will be processed as outlined in Sections C and D.

C. Test Material Simulation of Consumer Use (20 x wash/drying condition):

In order to simulate consumer use and to prove the efficacy of the product over a prolonged use period, which will include environmental stressing and wash/drying exposures, the fabric carriers will be processed as follows:

1. Pre-conditioning (applicable prior to the first wash/drying cycle only): Fabric carriers will be incubated at 36±2°C, 85-100% RH for 24 hours followed by UV irradiation at room temperature (20-25°C) in a biological safety cabinet for one hour before the initial fungal challenge as outlined in Step 2, part b below.

2. Environmental stressing conditions:

   a. Fabric carriers will be dried at 36±2°C, 85-100% RH for two hours and UV irradiation at room temperature (20-25°C) in a biological safety cabinet for 15 min to mimic wear and tear (applicable to wash/drying cycles 2 – 20 only)

   b. Fungal challenge: Fabric carriers will be inoculated using an inoculum containing synthetic sweat as organic soil with a final concentration of $10^2 - 10^3$ conidia/mL. Each carrier will be inoculated with a 0.1 mL aliquot of the prepared inoculum. All fabric carrier types (test and control) may be inoculated on either side (surface).

   c. Fabric carriers will be dried at room temperature (20-25°C) for 20-30 minutes

3. Wash condition: Fabric carriers will be exposed to 80±15°C water containing a commonly used detergent, such as Woolite®, and bleach for 10 - 12 minutes.

4. Rinse condition: Fabric carriers will be thoroughly rinsed to assure that there is no residual detergent (Section D and Appendix I).

5. Drying condition: Fabric carriers will be tumble dried at 175±30°F (62-96°C) for 20 - 30 minutes (Section D and Appendix I).

6. Repeat steps 2 – 5 until the completion of 20 cycles

7. After 20 complete cycles, the fabric carriers will be processed as detailed in the Fungicidal Efficacy Test section (Section E).

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D. Fabric Carrier Washing and Drying (20 x wash/drying condition):

The simulated washing cycles will be conducted using a modification of the Petrocci and Clarke method published in JOAC, 1981[4], as well as the ASTM[5] method E2274-03, "Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants" for the evaluation of laundry products for use in top-load or standard laundering operations and ASTM method E2406, "Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use in High Efficiency Washing Operations".

An "efficacy indicator" is not available for this product. A worst case washing condition will include exposing the fabric carriers to 80±15°C hot water containing a strong, commonly used detergent such as Woolite® mixed with bleach.

The fabric carriers will then be thoroughly rinsed with sterile tap water until it is assured that there is no residual detergent solution that may interfere is with the ionic release or interfere with the fungal assay itself.

After each wash cycle the fabric carriers will be tumble dried at 175±30°F (62-96°C) for 20 - 30 minutes. AM

See Appendix I for details regarding the specific procedures for the wash and drying procedure.

Once the regimen has been conducted for 20 complete cycles, the fabric carriers will be exposed to UV irradiation at room temperature (20-25°C) in a biological safety cabinet for 30 minutes per side followed by air drying at room temperature (20-25°C) for at least 30 minutes. The fabric carriers will then be transferred to sterile Petri dishes before the initiation of the Fungicidal Efficacy Test (Section E).

[4] Petrocci and Clarke, J. Association of Official Analytical Chemists (AOAC), 52:836-842.
[5] ASTM: American Society of Test materials

E. Fungicidal Efficacy Test (performed using both conditions)

Five replicate fabric carriers per lot of the treated test and untreated control fabric will be evaluated in this study using three lots of the test fabric and one lot of control fabric. One exposure time will be evaluated for each replicate fabric carrier with the aim of showing greater than an average 3-log (99.9%) reduction in twelve hours of the treated test fabric over the untreated control fabric.

The following procedures will be performed in two independent phases whereas the initial phase will include the evaluation of the 0 wash fabric carriers will be tested without being subjected to the simulated environmental stressing or the wash/drying cycle regimen.

The secondary phase will be performed using fabric carriers which have been subjected to the 20 x wash/drying condition.

A 0.1 mL aliquot of a high-level culture preparation (containing 10^5 – 10^7 conidia/mL) will be used to inoculate each fabric carrier by pipetting across the area of each fabric carrier ensuring consistent distribution across all fabric carriers. All fabric carriers (test and control) may be inoculated on either side (surface).

The inoculated fabric carriers will be incubated as soon as possible post-inoculation at 36±2°C, 85-100% RH until the conclusion of the twelve hour contact time in open Petri dishes. The contact time will be initiated immediately after inoculation.

Upon completion of the contact time, each fabric carrier will be aseptically transferred to a sterile stomacher bag containing 100 mL of Neutralizer and stomached for approximately five minutes. Post-stomaching, duplicate 0.1 mL, 1.0 mL and 10.0 mL aliquots will be removed from the bag and transferred into independent tubes containing Neutralizer to yield a final volume of 20 mL per tube. Each sample tube will be filtered in its entirety through a 0.45 µm membrane and then the filter membrane will be rinsed using approximately 40 mL of the Neutralizer. The filter membranes will be removed and placed onto individual NGA plates. All plates will be inverted and incubated at 25-30°C for 3-5 days.

F. Controls

1. Neutralizer effectiveness control:

This control will be included to demonstrate absence of residual antimicrobial activity post neutralization.

For each lot of treated test fabric, a single carrier will be subcultured into a sterile stomacher bag containing 100 mL of the Neutralizer and stomached for approximately five minutes.

Post-stomaching, duplicate 0.1 mL, 1.0 mL and 10.0 mL aliquots will be removed from the bag and transferred into independent tubes containing the neutralizer to yield a final volume of 20 mL per tube. Each sample tube will be filtered through a 0.45 µm membrane and the filter apparatus will be deactivated. Approximately 40 mL of the Neutralizer will be added to the filter cup containing the filter membrane and fewer than 100 conidia of the challenge microorganism will be added to the Neutralizer. The filtration apparatus will be activated to draw the Neutralizer containing fewer than 100 CFU conidia through the filter membrane. The membrane filters will be removed and placed onto individual NGA plates.

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The count of the fungi added to the Neutralizer will be confirmed via standard filtration technique. Duplicate samples of the selected aliquot will be individually processed. The membrane filters will be placed onto individual NGA plates. All plates will be incubated with the test.

2. High-level and Low-level Inoculation Confirmation:

Each day that inoculations are performed during the 20 x wash/drying regimen (low-level inoculum), as well as on each day of the fungicidal efficacy evaluation (high-level inoculum), the CFU/mL will be confirmed by serially diluting the preparation using PBDW and plating selected aliquots from appropriate dilutions using duplicate NGA spread plates. All plates will be inverted and incubated at 25-30°C for 3-5 days.

3. Sterility controls

On the day of each fungicidal efficacy evaluation, duplicate 0.1 mL aliquots of Neutralizer and PBDW will be plated using independent NGA spread plates. These plates, along with duplicate NGA plates will be incubated with the test.

4. Confirmation of the challenge microorganism:

On the day of plate readings for each fungicidal efficacy evaluation (0 wash and 20 x wash/drying conditions), one isolated colony from an untreated control fabric plate will be examined visually for colony morphology and wet preps observed to confirm identity. An isolated colony from a treated test fabric plate (if applicable) will be treated in the same manner and compared to the untreated control fabric prep. The results will be reported in the final report.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- Neutralizer must be proven to be effective whereas the difference between the confirmed CFU added is within 0.3-Log of the recovered CFU for the treated fabric samples.
- The untreated control fabric counts must average $\geq 1.0 \times 10^4$ CFU/carrier
- All sterility controls must be negative for growth.

PRODUCT EVALUATION CRITERIA:

To meet the proposed effectiveness requirements, the average CFU/carrier recovered for the treated test fabric must achieve a three-log reduction (99.9%) in viable microorganisms over the average CFU/carrier recovered for the control fabric is required.

CONFIDENTIALITY:

All data generated at MICROBIOTEST are held in strictest confidence and are available only to the sponsor. In turn, no reference to the work, data, or MICROBIOTEST may be made public without the written consent of MICROBIOTEST..

REPORT FORMAT:

MICROBIOTEST employs a standard report format for each test design. Each final report will provide the following information:

- Sponsor identification
- Test material identification
- Type of assay and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Test results presented in tabular form
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion
- Signed Quality Assurance and Compliance Statements

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PERSONNEL AND TESTING FACILITIES:

A study director will be assigned prior to initiation of the test. Resumes are maintained and are available on request. This study will be conducted at MICROBIOTEST.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test material records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test material; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately. The date the study director signs the protocol will be the initiation date. All project sheets will be forwarded to the study sponsor.

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Table 2: SUMMARY OF PRIMARY TEST AND CONTROL SAMPLES TO BE ASSAYED

Parameter	Fabric	Cycles*	Sample Designation
1	Test Fabric, Lot # 1	0	Test Fabric, Lot # 1, 0 wash condition, 5 replicates
2	Test Fabric, Lot # 2	0	Test Fabric, Lot # 2, 0 wash condition, 5 replicates
3	Test Fabric, Lot # 3	0	Test Fabric, Lot # 3, 0 wash condition, 5 replicates
4	Control Fabric	0	Control Fabric, 0 wash condition, 5 replicates
5	Test Fabric, Lot # 1	0	Neutralizer Effectiveness Control, 1 replicate
6	Test Fabric, Lot # 2	0	Neutralizer Effectiveness Control, 1 replicate
7	Test Fabric, Lot # 3	0	Neutralizer Effectiveness Control, 1 replicate
8	Test Fabric, Lot # 1	20	Test Fabric, Lot # 1, 20 x wash/drying condition, 5 replicates
9	Test Fabric, Lot # 2	20	Test Fabric, Lot # 2, 20 x wash/drying condition, 5 replicates
10	Test Fabric, Lot # 3	20	Test Fabric, Lot # 3, 20 x wash/drying condition, 5 replicates
11	Control Fabric	20	Control Fabric, 20 x wash/drying condition, 5 replicates

*Represents wash/drying and simulated environmental stressing procedures.

MISCELLANEOUS INFORMATION: The following information is to be completed by sponsor before initiation of study:

A. Name and address: Cupron Inc.
Suite 123
800 East Leigh Street
Richmond, VA 23219

B. Test fabric: 75% Cupron Polyester / 25% Nylon

Active ingredient: Copper oxide

Lot No 1: 501
Lot No 2: 502
Lot No 3: 503

C. Control fabric:

Lot No. 500

D. Fungicidal efficacy evaluation conditions:

Contact time: 12 hours

Environmental: 36±2°C @ 85-100% RH

E. Precautions/storage conditions – see MSDS or Certificate of Analysis
☐ provided ■ not provided

Note: all Test and Control fabrics were manufactured on 02/06/11.

REPORT HANDLING: The sponsor intends to submit this information to: ■ US EPA ☐ US FDA ☐ Health Canada ☐ CAL DPR ☐ ARTG ☐ other: Internal Purposes

STUDY CONDUCT: ■ GLP ☐ non-GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 2/27/13
Alastair B. Monk, PhD

Study Director Signature: _____ Date: 02/28/13
Angela L. Hollingsworth

APPENDIX I

Simulation of Washing and Rinsing Fabric

Wash and rinse cycle:

Detergent and bleach solution preparation: The detergent (Woolite or equivalent) and bleach will be diluted using 80±15°C sterile tap water as directed by the label of the products for standard laundering applications.

The prepared detergent solution* will be added to the exposure chambers [Mason (or equivalent) jars] in 60 mL portions per chamber. The fabric carriers will be added to the exposure chambers containing the detergent solution (each five replicate set will be maintained in independent jars; three test sets and one untreated control set for a total of four jars).

The exposure chambers will be placed into a laundrometer (a device that will be used to simulate tumble-wash; the tumbling action will be maintained at 40-60 RPM) and the device will be activated for 10 - 12 minutes. The tumbling device will be inactivated and the detergent solution will be discarded from the exposure chambers without removing the fabric carriers.

The fabric carriers will then be thoroughly rinsed until it is assured that there is no residual detergent solution that may interfere with the ionic release or interfere with the fungal assay itself. Sterile tap water (maintained at room temperature, 20-25°C) will be used for the rinse cycle. At least 60 mL of tap water will be added to each exposure chamber containing the fabric carriers. The exposure chambers will be placed into the laundrometer and the device will be activated for five - seven minutes (with the tumbling action maintained at 40-60 RPM).

After rinsing, the fabric carriers will be removed from the exposure chambers and blotted dry by pressing between sterile paper towels.

*Note: for detergent solution preparation: a simulated "small load" for a standard washer (approximately 13-Gallons, or 49,205 mL). For the Woolite detergent, the volume recommended for a small load is approximately 20 mL. For the bleach, ¼ cup, or 177.4 mL is recommended. For each wash cycle, 492 mL of tap water was warmed to the designated temperature and mixed with 0.2 mL of Woolite detergent and 1.8 mL of bleach. From this preparation, 60 mL aliquots were dispensed as required.

APPENDIX I (continued)

Simulation of Washing and Rinsing Fabric

Tumble drying cycle:

The fabric carriers will be placed into individual laundering bags (one bag for each group of five replicate carriers for manipulation purposes) and tumble dried for 20 - 30 minutes at 175±30°F (62-96°C).

The test and control fabric carriers will be dried in independent dry cycles however the test fabric carriers for each of the three test lots may be dried together.

Date Issued: 02/28/13 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 619-135

STUDY TITLE: Assessment of Fungicidal Efficacy of Copper Oxide Impregnated Fabric After Twelve Hours of Exposure - *Trichophyton mentagrophytes*	**STUDY DIRECTOR:** Angela L. Hollingsworth 02/28/13 Signature Date

TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO:**
75% Cupron Polyester / 25% Nylon	501	02/26/13	D107
75% Cupron Polyester / 25% Nylon	502	02/26/13	D108
75% Cupron Polyester / 25% Nylon	503	02/26/13	D109
Control Fabric (75% Polyester / 25% Nylon Untreated)	500	02/26/13	D106

PERFORMING DEPARTMENT: Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: G6 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ☐Yes / ■ No

PHYSICAL DESCRIPTION: ☐ Solid ☐ Liquid ☐ Aerosol ■ Other: Fabric

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 02/28/13 **TERMINATION DATE:** 03/19/13

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other: Internal Purposes

SPONSOR: Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Alastair B. Monk, PhD Phone: 804-381-5514 E-mail: amonk@cupron.com

TEST CONDITIONS:

Challenge microorganism:	*Trichophyton mentagrophytes*, ATCC 9533
Active ingredient:	Copper oxide
Neutralizer:	Letheen Broth – 2X
Contact Time:	12 hours
Contact Temperature:	36±2°C
Environmental Condition:	85-100% RH
Inoculum carrier:	Synthetic sweat (acidic)
Incubation Time(s):	3-5 days
Incubation Temperature(s):	25-30°C

Comments: This test will be conducted using two independent sets of fabric carriers whereas one set will undergo the fungicidal efficacy evaluation based on a 0 wash regimen and the secondary set will undergo the efficacy evaluation after a 20 x wash/drying regimen.

Note: all Test and Control fabrics were manufactured on 02/06/11.

Date Issued: 03/25/13 Project Sheet No. 2 Page No. 1	Laboratory Project Identification No. 619-135		
STUDY TITLE: Assessment of Fungicidal Efficacy of Copper Oxide Impregnated Fabric After Twelve Hours of Exposure - *Trichophyton mentagrophytes*	**STUDY DIRECTOR:** Angela L. Hollingsworth Signature 03/25/13 Date		
TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO:**
75% Cupron Polyester / 25% Nylon	501	02/26/13	D107
75% Cupron Polyester / 25% Nylon	502	02/26/13	D108
75% Cupron Polyester / 25% Nylon	503	02/26/13	D109
Control Fabric (75% Polyester / 25% Nylon Untreated)	500	02/26/13	D106
PERFORMING DEPARTMENT: Applied Microbiology Laboratory	**STORAGE CONDITIONS:** Location: G6 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other: Internal Purposes			
SPONSOR: Cupron Inc. 800 East Leigh Street, Suite 123 Richmond, VA 23219	**CONTACT PERSON:** Alastair B. Monk, PhD Phone: 804-381-5514 E-mail: amonk@cupron.com		

EXPLANATION:

Protocol Amendment(s):

1. The protocol inadvertently describes the temperature range for the tumble drying conditions required for the Fabric Carrier Washing and Drying component as 175±30°F (62-96°C). The temperature range intended was 62-96°C. All references to 175±30°F should be disregarded. This amendment serves to correct a typographical error in the protocol.

2. For all references to the manufacture date of the three lots of the treated fabric and the single lot of the control fabric; the date was 02/06/12, not 02/06/11. This amendment serves to correct a typographical error in the protocol as well the initial project sheet.