MycoScience
Specialists in Microbiology & Regulatory Affairs

ANTIMICROBIAL EFFICACY
TIME KILL STUDY

CLIENT: Silvex Inc.
45 Thomas Drive
Westbrook ME 04092

TEST#: 14-0575 SIL

DATE: 05/28/14

TEST ARTICLES:

Used Silver Coated Flatware:

(1) Dinner Fork, (1) Salad Fork, (1) Knife, (1) Tablespoon

CONTROL ARTICLES:

Non-Silver Coated Flatware:

(1) Dinner Fork, (1) Salad Fork, (1) Knife, (1) Tablespoon

TEST REQUESTED:

Antimicrobial Efficacy – Time Kill Study

TEST OBJECTIVE:

The purpose of this study was to determine how rapidly and effectively over time the used silver coated flatware kills or prevents the growth of microorganisms in comparison to non-silver coated flatware.

TEST SYSTEM:

The test articles were evaluated for microbial kill via Time Kill methodology. Samples were tested at 1 hour, 3 hours, 5 hours and 24 hours contact versus the microorganisms listed below.

The test microorganisms used in this study were:

1) *Staphylococcus aureus* ATCC # 6538 (Bacteria)
2) *Pseudomonas aeruginosa* ATCC # 9027 (Bacteria)
3) *Escherichia coli* ATCC # 8739 (Bacteria)
4) *Candida albicans* ATCC # 10231 (Yeast)

The stock cultures were maintained per current AOAC methodology for disinfectant testing. Purity of the culture was determined by macroscopic and microscopic morphology.

TEST PROCEDURE:

Reference Mycoscience Protocol: "Silvex Inc. Antimicrobial Efficacy - Time Kill Study Silver Coated Flatware Mycoscience Labs Study Protocol 7/11/13" (Modified For Various Flatware)

1.0 Test Microorganism Suspension Preparation

1.1 The *S. aureus, P. aeruginosa,* and *E. coli* bacterial cultures were transferred to Trypticase Soy Agar (TSA) plates and incubated at 35 - 37°C for 18 - 24 hours. A minimum of one daily transfer was made. The cultures were harvested from the surface of a TSA plate and were suspended in sterile 0.9% saline. Further serial dilutions were performed as necessary, and each microorganism final suspension was standardized with a spectrometer to a concentration of ~5.0×10^7 CFU/mL.

1.2 The *C. albicans* yeast culture was transferred to a Sabouraud Dextrose Agar (SDA) plate with incubation at 30 – 35°C for 24 hours. A minimum of one daily transfer was made with the final transfer to SDA with incubation for 24 - 48 hours at 30 - 35°C. The culture was harvested from the surface of a SDA plate and was suspended in sterile 0.9% saline. Further serial dilutions were performed as necessary and the final suspension was standardized with a spectrometer to a concentration of ~5.0×10^7 CFU/mL. The final concentration was verified by standard dilution and plate count methodology, with incubation as in Sect. 3.0 below.

2.0 Sample Preparation

Each sample flatware piece and control flatware piece (silver coated and non-coated) were aseptically transferred to a sterile vessel for each test microorganism. A sample and control flatware was tested for each test microorganism at each time point. 100mL of sterile purified deionized (DI) water was added to each sterile vessel via sterile pipet. Samples were held at 20 - 25°C throughout the test.

3.0 Time Kill Assay

The duplicate sample and control vessels were inoculated with 1.0mL of the prepared microorganism suspension. Exposure time intervals consisted of 1 hour, 3 hours, 5 hours, and 24 hours. The sample vessels were held at 20 - 25°C after inoculation. At each exposure time interval, the sample vessels were mixed thoroughly, and 1.0mL was removed and transferred to 9mL of Letheen Broth. Serial dilutions (1:10) were performed in 9mL Letheen Broth and 0.1mL aliquots were plated via spread plate to the surface TSA plates containing neutralizers (0.1% Tween 80 and 0.05% Lecithin) for bacterial recovery or SDA plates containing neutralizers (0.1% Tween 80 and 0.05% Lecithin) for fungal (yeast) recovery. Additionally, 0.1mL was also removed and plated directly to the surface of TSA or SDA w/ neutralizers. The TSA plates were incubated at 35 -37°C for 48 -72 hours (bacteria), and the SDA at 30 – 35°C for 48-72 hours (yeast), and then were enumerated.

Negative controls were run on all sterile media and materials with incubation as above.

4.0 Neutralization Verification

Refer to report 13-1448 SIL for the neutralization verification. Comparable growth on sample and control plates after incubation confirmed neutralizer effectiveness.

RESULTS:

See attached Tables.

CONCLUSIONS:

The data demonstrates a distinct difference in the reduction of microorganisms in the presence of the used silver coated flatware samples over time in comparison to the non-coated control flatware samples for the microorganisms tested. This indicates the used silver coated flatware is still demonstrating antimicrobial properties after repeated use.

A 8.40% reduction at 1 hour and a 76.20% reduction at 5 hours of *Staphylococcus aureus* was seen for the silver coated flatware compared to the non-coated flatware.

A 98.00% reduction at 1 hour and a 99.95% reduction at 5 hours of *Pseudomonas aeruginosa* was seen for the silver coated flatware compared to the non-coated flatware.

A 86.90% reduction at 1 hour and a 99.92% reduction at 24 hours of *Escherichia coli* was seen for the silver coated flatware compared to the non-coated flatware.

A 22.06% reduction at 5 hours and a 17.46% reduction at 24 hours of *Candida albicans* was seen the silver coated flatware compared to the non-coated flatware.

Analyst/s: Amy Cameron Date: 5/28/14

Reviewed By: R. Arsenault Date: 5/28/14

RESULTS:

Table 1:

Antimicrobial Efficacy of Used Silver Coated Flatware in Comparison to Non-Silver Coated Flatware

***Staphylococcus aureus* ATCC # 6538**

Time Point	Dinner Fork	Recovered CFU/mL	Mean Microorganism Log Reduction	Microorganism % Reduction
1 HOUR	Silver Coated	1.09×10^6	0.04	8.40%
	Non-Silver Control	1.19×10^6	N/A	N/A
3 HOURS	Silver Coated	7.6×10^5	0.16	30.28%
	Non-Silver Control	1.09×10^6	N/A	N/A
5 HOURS	Silver Coated	1.69×10^5	0.62	76.20%
	Non-Silver Control	7.1×10^5	N/A	N/A
24 HOURS	Silver Coated	<10	4.06	99.99%
	Non-Silver Control	1.16×10^5	N/A	N/A

RESULTS:

Table 2:

Antimicrobial Efficacy of Used Silver Coated Flatware in Comparison to Non-Silver Coated Flatware

***Pseudomonas aeruginosa* ATCC # 9027**

Time Point	Salad Fork	Recovered CFU/mL	Mean Microorganism Log Reduction	Microorganism % Reduction
1 HOUR	Silver Coated	1.0×10^4	1.70	98.00%
	Non-Silver Control	5.0×10^5	N/A	N/A
3 HOURS	Silver Coated	1.3×10^3	2.32	99.52%
	Non-Silver Control	2.7×10^5	N/A	N/A
5 HOURS	Silver Coated	30	3.47	99.95%
	Non-Silver Control	8.8×10^4	N/A	N/A
24 HOURS	Silver Coated	10	0.70	80.00%
	Non-Silver Control	50	N/A	N/A

RESULTS:

Table 3:

Antimicrobial Efficacy of Used Silver Coated Flatware in Comparison to Non-Silver Coated Flatware

***Escherichia coli* ATCC # 8739**

Time Point	Knife	Recovered CFU/mL	Mean Microorganism Log Reduction	Microorganism % Reduction
1 HOUR	Silver Coated	1.10×10^5	0.88	86.90%
	Non-Silver Control	8.4×10^5	N/A	N/A
3 HOURS	Silver Coated	5.7×10^4	0.86	86.10%
	Non-Silver Control	4.1×10^5	N/A	N/A
5 HOURS	Silver Coated	1.0×10^3	2.41	99.61%
	Non-Silver Control	2.55×10^5	N/A	N/A
24 HOURS	Silver Coated	<10	3.12	99.92%
	Non-Silver Control	1.33×10^4	N/A	N/A

RESULTS:

Table 4:

Antimicrobial Efficacy of Used Silver Coated Flatware in Comparison to Non-Silver Coated Flatware

***Candida albicans* ATCC # 10231**

Time Point	Tablespoon	Recovered CFU/mL	Mean Microorganism Log Reduction	Microorganism % Reduction
1 HOUR	Silver Coated	6.2×10^5	0.08	16.22%
	Non-Silver Control	7.4×10^5	N/A	N/A
3 HOURS	Silver Coated	6.4×10^5	0.00	0%
	Non-Silver Control	6.3×10^5	N/A	N/A
5 HOURS	Silver Coated	5.3×10^5	0.11	22.06%
	Non-Silver Control	6.8×10^5	N/A	N/A
24 HOURS	Silver Coated	5.2×10^5	0.08	17.46%
	Non-Silver Control	6.3×10^5	N/A	N/A