



October 31, 2008

Mr. Howard Ohlhausen
Director
Unelko Corporation
14641 N. 74th Street
Scottsdale, AZ 85260

**RE: Sani-Shield Product Testing
Zone of Inhibition Study
LEGEND Project No. 0810-02**

Dear Mr. Ohlhausen:

1.0 INTRODUCTION AND BACKGROUND

On October 14, 2008, Legend Technical Services, Inc. (LEGEND) initiated a "Zone of Inhibition" study as a means of testing the Unelko Product, Sani-Shield 3-in-1 Surface Care (also marketed as Clean & Shield Surface Care and Clean & Shield Bathroom Care). In this study, glass microscope slides coated with Sani-Shield were placed on culture plates of selected bacteria (*Enterobacter aerogenes*) and fungi (*Aspergillus niger*) and observed for a zone of inhibition around the treated slides. Results were reported as the presence or absence of a zone of inhibition, and the zone was reported in millimeters, if observed.

2.0 STUDY OVERVIEW

Study Preparation

LEGEND prepared cultures of *Aspergillus niger* fungi and *Enterobacter aerogenes* bacteria obtained from the American Type Culture Collection (ATCC). *Aspergillus niger* was cultured on Potato Dextrose Agar (PDA), and *Enterobacter aerogenes* was cultured on Trypticase Soy Agar with 5% Sheep Blood (SBA).

Glass microscope slides treated/coated with Sani-Shield were supplied by the Unelko Group.

Test Procedure

Following preparation of bacterial and fungal cultures, suspensions of the organisms were prepared as follows:

Suspension A – 10 mL of a sterile buffered water solution containing Tween 80 (surfactant) was applied to the surface of a mature culture plate of *A. niger*. The surface was agitated using a sterile plastic spreader to remove surface spores. A pipet was used to withdraw 1 mL aliquots of the spore suspension, which were applied to three separate plates of PDA.

Suspension B – 10 mL of a sterile buffered water solution was applied to the surface of a mature culture plate of *E. aerogenes*. The surface was agitated using a sterile spreader to remove cells of bacteria. A pipet was used to withdraw 1 mL aliquots of bacterial suspension, which were applied to three separate plates of SBA.

Using the prepared glass microscope slides provided by Unelko, the following samples were prepared:

Sample 1 – PDA plate, *A. niger*, fully treated glass slide

Sample 2 – PDA plate, *A. niger*, glass slide treated only on top and edges

Sample 3 – PDA plate, *A. niger*, untreated glass slide (Control)

Sample 4 – PDA plate, sterile (Media blank)

Sample 5 – SBA plate, *E. aerogenes*, fully treated glass slide

Sample 6 – SBA plate, *E. aerogenes*, glass slide treated only on top and edges

Sample 7 – SBA plate, *E. aerogenes*, untreated glass slide (Control)

Sample 8 – SBA plate, sterile (Media blank)

After preparation, each of the PDA plates was incubated at 25°C for at least 7 days. SBA plates were incubated at 35°C for 48 hours.

Following incubation, each of the cultures was examined for a zone of inhibition of growth around the glass microscope slides. Media blanks were checked to confirm the absence of growth. If present, the zone of inhibition was measured by the analyst and reported in units of millimeters.

3.0 ANALYTICAL RESULTS

Tables 1 and 2 include the analytical results for the fungi and bacteria groups, respectively:

Table 1: Fungi Results – *Aspergillus niger*

Sample Identification	Media	Zone of Inhibition (P/A)*
1) Fully Treated Slide	PDA	Absent
2) Slide Treated on Top and Edges	PDA	Absent
3) Untreated Slide (Control)	PDA	Absent
4) Media Blank	PDA	No Growth (Sterile)

Table 2: Bacteria Results – *Enterobacter aerogenes*

Sample Identification	Media	Zone of Inhibition (P/A)*
5) Fully Treated Slide	SBA	Absent
6) Slide Treated on Top and Edges	SBA	Absent
7) Untreated Slide (Control)	SBA	Absent
8) Media Blank	SBA	No Growth (Sterile)

* P/A = Presence/Absence

4.0 DISCUSSION AND CONCLUSIONS

After the required incubation periods, the examined plates containing the test slides exhibited no zones of inhibition for either the *Aspergillus niger* or *Enterobacter aerogenes*. There was no difference between the observed results of the cultures containing the Sani-Shield treated slides and those of the control. These results demonstrate that Sani-Shield, when applied to a hard surface, is restricted to that surface and does not leach into the surrounding medium.

5.0 REMARKS

If there are any additional questions regarding the final results or the methodology used for this project, or if further testing is elected please contact LEGEND at (602) 324-6100.

Prepared By:
LEGEND TECHNICAL SERVICES OF ARIZONA, INC.



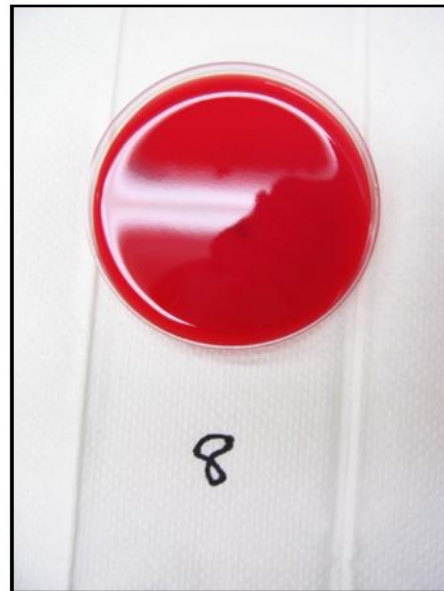
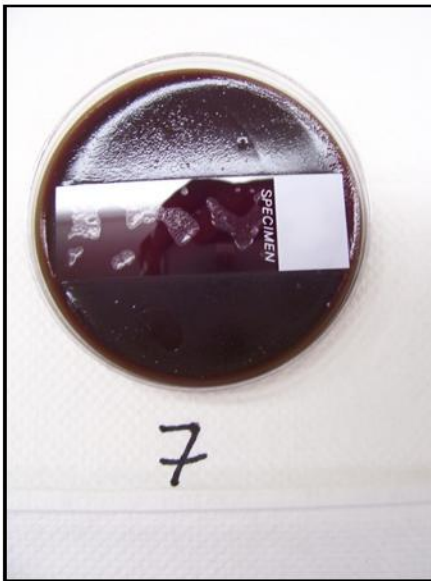
Robert M. Vertefeuille
Director of Operations/Senior Microbiologist

APPENDIX A: FUNGI PHOTOS—ZONE OF INHIBITION STUDY



Aspergillus niger

APPENDIX B: BACTERIA PHOTOS—ZONE OF INHIBITION STUDY



Enterobacter aerogenes