



February 29, 2008

Mr Tom Kraus
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34655 Mills Road,
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OH 44039

**Antimicrobial Assessment of Five Belt Samples
from Bioplastics**

2402457

Five belt samples, four of which were treated with either Ultra-Fresh CA-16 or Ultra-Fresh 95, were received from Bioplastics on January 30, 2008. At Thomson Research Associates Inc., the samples were tested for antimicrobial activity against the *Escherichia coli* test organism using a quantitative test method.

PROCEDURE

Quantitative Antibacterial Assessment:

ISO 22196:2007 (JIS Z 2801:2006) was used to quantitatively test the specimen for antibacterial activity. In brief:

1. The sample was placed into a container with a lid.
2. A 0.4 mL inoculum of *Escherichia coli* (ATCC #8739) was placed, in microdroplets, on the surface of the samples (24 hour culture, approx 2.88×10^5 organisms/mL). Sterile films were placed over the inoculum to encourage good contact.
3. The specimen was incubated 24 hours at 37C.
4. 50 mL of Lethen broth was added to the container and shook. The liquid was plated using dilution techniques.
5. The "Value of Antimicrobial Activity" was carried out using the formula

$$R = [\log (B/C)]$$

Where R = value of antimicrobial activity

B = Average of the number of viable cells of bacteria on the untreated test piece after 24 hours

C = Average of the number of viable cells of bacteria on the antimicrobial test piece after 24 hours.

THOMSON RESEARCH ASSOCIATES INC.

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RESULTS

Quantitative Assessment of Activity - ISO 22196:2007 (JIS Z 2801:2006) <i>E. coli</i>					
Concentration of inoculum (M_a)				log 2.88×10^5 CFU/mL	
Sample Description		No. Bacteria Recovered	Log Recovery	R = [log(B/C)]	% Survival
1	B05YY200YE417-with Ultra-Fresh EVA-95	1.01×10^7	7.0	0.1	82.3%
2	B05YY200YE417-with Ultra-Fresh PE CA-16	2.60×10^4	4.4	2.7	0.2%
3	B05UT100BL231-with Ultra-Fresh EVA-95	1.41×10^7	7.1	0.0	100.0%
4	B05UT100BL231-with Ultra-Fresh PE CA-16	4.44×10^6	6.6	0.5	36.1%
5	B05YY200YE417-Control with no additive	1.23×10^7	7.1	----	----

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February 29, 2008

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**Antimicrobial Assessment of Five Belt Samples
from Bioplastics**

2402459

Five belt samples, four of which were treated with either Ultra-Fresh CA-16 or Ultra-Fresh 95, were received from Bioplastics on January 30, 2008. At Thomson Research Associates Inc., the samples were tested for antimicrobial activity against the Methicillin Resistant *Staphylococcus aureus* test organism using a quantitative test method.

PROCEDURE

Quantitative Antibacterial Assessment:

ISO 22196:2007 (JIS Z 2801:2006) was used to quantitatively test the specimen for antibacterial activity. In brief:

1. The sample was placed into a container with a lid.
2. A 0.4 mL inoculum of Methicillin Resistant *Staphylococcus aureus* (ATCC #33591) was placed, in microdroplets, on the surface of the samples (24 hour culture, approx 2.78×10^5 organisms/mL). Sterile films were placed over the inoculum to encourage good contact.
3. The specimen was incubated 24 hours at 37C.
4. 50 mL of Lethen broth was added to the container and shook. The liquid was plated using dilution techniques.
5. The "Value of Antimicrobial Activity" was carried out using the formula

$$R = [\log (B/C)]$$

Where R = value of antimicrobial activity

B = Average of the number of viable cells of bacteria on the untreated test piece after 24 hours

C = Average of the number of viable cells of bacteria on the antimicrobial test piece after 24 hours.

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RESULTS

Quantitative Assessment of Activity - ISO 22196:2007 (JIS Z 2801:2006)					
MRSA					
Concentration of inoculum (M_a)				log 2.78×10^5 CFU/mL	
Sample Description		No. Bacteria Recovered	Log Recovery	R = [log(B/C)]	% Survival
1	B05YY200YE417-with Ultra-Fresh EVA-95	$<5.00 \times 10^1$	<1.7	>3.9	<0.1%
2	B05YY200YE417-with Ultra-Fresh PE CA-16	3.00×10^2	2.5	3.1	<0.1%
3	B05UT100BL231-with Ultra-Fresh EVA-95	1.70×10^3	3.2	2.4	0.4%
4	B05UT100BL231-with Ultra-Fresh PE CA-16	4.78×10^3	3.7	1.9	1.1%
5	B05YY200YE417-Control with no additive	4.13×10^5	5.6	----	----

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February 08, 2008

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Antimicrobial Assessment of Five Belt Samples
from Bioplastics

2402455

Five belt samples, which were treated with Ultra-Fresh CA-16 and Ultra-Fresh 95, were received from Bioplastics on January 30, 2008. At Thomson Research Associates Inc., the samples were tested for antimicrobial activity against the *Staphylococcus aureus* test organism using a Japanese quantitative test method.

PROCEDURE

Quantitative Antibacterial Assessment:

ISO 22196:2007 (E) (JIS Z 2801:2006) was used to quantitatively test the specimen for antibacterial activity. In brief:

1. The sample was placed into a container with a lid.
2. A 0.4 mL inoculum of *Staphylococcus aureus* (ATCC #6538) was placed, in microdroplets, on the surface of the samples (24 hour culture, approx 3.32×10^5 organisms/mL). Sterile films were placed over the inoculum to encourage good contact.
3. The specimen was incubated 24 hours at 37C.
4. 50 mL of phosphate buffer was added to the container and shook. The liquid was plated using dilution techniques.
5. The "Value of Antimicrobial Activity" was carried out using the formula

$$R = [\log (B/C)]$$

Where R: value of antimicrobial activity

B = Average of the number of viable cells of bacterial on the untreated test piece after 24 hours

C = Average of the number of viable cells of bacteria on the antimicrobial test piece after 24 hours.

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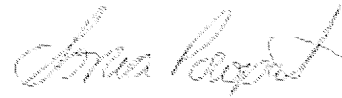
RESULTS

Quantitative Assessment of Activity - JIS Z 2801:2006 <i>S. aureus</i>					
Concentration of inoculum (M _a)				log 3.32 x 10 ⁵ CFU/mL	
Sample Description		No. Bacteria Recovered	Log Recovery	R = [log(B/C)]	% Survival
1	B05YY200YE417-with Ultra-Fresh EVA-95	2.07 x 10 ⁴	4.3	1.7	2.3%
2	B05YY200YE417-with Ultra-Fresh PE CA-16	4.57 x 10 ⁴	4.7	1.3	5.0%
3	B05UT100BL231-with Ultra-Fresh EVA-95	3.42 x 10 ⁴	4.5	1.5	3.8%
4	B05UT100BL231-with Ultra-Fresh PE CA-16	1.37 x 10 ³	3.1	2.9	0.1%
5	B05YY200YE417-Control with no additive	9.12 x 10 ⁵	6.0	----	----

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