



TECHNICAL REPORT

For

ViaClean Technologies, LLC
230 South Broad Street
Suite 1201
Philadelphia, Pennsylvania 19102

ViaClean Technologies, LLC
1406 East Pine Street
Midland, Michigan 48640

ASTM E2149 - 2013
Determining the Antimicrobial Activity of Immobilized
Antimicrobial Agents Under Dynamic Contact Conditions

Antimicrobial Activity against *Escherichia coli*
Treated Fabrics in "Shaker Test"

Analysis performed by

John Limbach
Microbiology Technical Director
Sanders Laboratories
1050 Endeavor Court
Nokomis, Florida 34275

Initiation Date: August 28, 2018
Completion Date: September 1, 2018
Sanders Laboratory Number 1808809

1. Materials Submitted for Testing:

Five untreated control fabrics and five corresponding treated fabrics were sent to Sanders Laboratories for ASTM E2149 testing. These fabrics are for the Paris Linens – ViaClean Laundry Trial, July 2018. This report covers the Escherichia coli resistance testing on the fabrics.

1. Bath Blanket - Control
2. Bath Blanket - Treated
3. Bed Sheet - Control
4. Bed Sheet – Treated
5. Towel – Control
6. Towel - Treated
7. Microfiber Sheet – Control
8. Microfiber Sheet – Treated
9. Incontinence Pad – Control
10. Incontinence Pad - Treated

2. Significance and Use:

This test method is designed to evaluate the resistance of non-leaching antimicrobial treated specimens to the growth of microbes under dynamic contact conditions. This dynamic shake flask test was developed for routine quality control and screening tests in order to overcome difficulties in using classical antimicrobial test methods to evaluate substrate-bound antimicrobials.

This test also allows for the versatility of testing contamination due to such things as hard water, proteins, blood, serum, various chemicals and other contaminants or physical/chemical stresses or manipulations of the specimens of interest. The antimicrobial activity of a substrate bound antimicrobial agent is dependent upon direct contact of microbes with the active chemical agent. This test determines the antimicrobial activity of treated specimens by shaking samples of surface bound materials in a concentrated bacterial suspension for a time period of 10 minutes to 24 hours as specified by the supplier of the samples.

- 2.1 Surface antimicrobial activity is determined by comparing results from the test samples to simultaneously run controls.
- 2.2 The presence of a leaching antimicrobial is both pre and post-determined by the presence of a zone of inhibition. ViaClean Technologies, LLC chose to eliminate the leaching test and washing test.
- 2.3 Stresses may include laundry, wear and abrasion, radiation and steam sterilization, UV exposure, solvent manipulation, temperature susceptibility or similar physical or chemical manipulation.

3. Preparation of Bacterial Inoculum.

Escherichia coli Strain (ATCC #25922) was grown on a Brain Heart Infusion Agar slant for 24 hours and collected in sterile Phosphate Buffer. 50 mL of the diluted culture in Phosphate Buffer was added to each of eleven 125 mL flasks.

4. Preparation of the Test Specimen:

A 2 inch by 2 inch square was cut from each fabric. Latex gloves were worn while handling the fabrics.

5. Testing Procedure:

The ten samples were each placed into separate 125 mL flasks each containing a 50 mL dilute suspension of *Escherichia coli* in Phosphate Buffer. Each flask was capped with aluminum foil and placed on the shaker. An initial “zero” contact time was established by plating out the control, flask #11, “inoculum only” diluted culture of *Escherichia coli* on 3M APC Petrifilm and incubating for 48 hours at 35° C. A 1 mL aliquot was taken from each of the eleven flasks after 24 hours on the shaker. Dilutions of 10⁻² and 10⁻⁴ were made and plated on Petrifilm for counts of the *Escherichia coli* that remained viable.

6. Evaluation of Results:

<u>Sample ID</u>	<u>cfu/mL Staph. (24 hr.)</u>	<u>% Kill</u>	<u>Antibacterial Activity</u>
1. Bath Blanket – Control	200,000	9.10%	0.04
2. Bath Blanket - Treated	92	99.96%	3.38
3. Bed Sheet – Control	100,000	54.54%	0.34
4. Bed Sheet – Treated	18,000	91.82%	1.08
5. Towel – Control	80,000	63.63%	0.44
6. Towel – Treated	240	99.89%	2.96
7. Microfiber Sheet – Control	480,000	no kill	0.00
8. Microfiber sheet – Treated	75	99.97%	3.46
9. Incontinence Pad – Control	49,000	77.73%	0.65
10. incontinence Pad – Treated	26	99.99%	3.93
11. Initial <i>Escherichia coli</i> count	220,000		
12. Final <i>Escherichia coli</i> count (after 24 hrs)	250,000	no kill	0.00

Calculation of the “Antibacterial Activity”: This is the difference in the logarithm of the viable cell count found on an antimicrobial-treated product and a control product after inoculation with, and incubation of, the bacteria. This is also known as the “Log Kill”

<u>Antibacterial Activity</u>	<u>%Kill compared to control</u>	<u>Comment</u>
<1.5	<96.8	poor
1.5 to 2.0	96.8 to 99.0	borderline
2.0 to 3.0	99.0 to 99.9	good
>3.0	>99.9	excellent

3. Conclusions:

- A. Four of the five treated fabrics showed excellent Antibacterial Activity against *Escherichia coli* following 24 hours on the shaker.
- B. The fifth treated fabric, bed sheet, had borderline Antibacterial Activity against *Escherichia coli* following 24 hours on the shaker. The bed sheet was thin and did not have the weight of the other samples.

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Determining the Antimicrobial Activity of Immobilized
Antimicrobial Agents Under Dynamic Contact Conditions

Antimicrobial Activity against *Staphylococcus aureus*
With Treated Fabrics in "Shaker Test"

Analysis performed by

John Limbach
Microbiology Technical Director
Sanders Laboratories
1050 Endeavor Court
Nokomis, Florida 34275

Initiation Date: August 27, 2018
Completion Date: August 31, 2018
Sanders Laboratory Number 1808955

1. Materials Submitted for Testing:

Three untreated control fabrics and three corresponding treated control fabrics were sent to Sanders Laboratories for ASTM E2149 testing. These fabrics are for the Paris Linens – ViaClean Laundry Trial, July 2018. This report covers the Staphylococcus aureus resistance testing on the fabrics.

- 3. Bed Sheet - Control
- 4. Bed Sheet – Treated
- 7. Microfiber Sheet – Control
- 8. Microfiber Sheet – Treated
- 9. Incontinence Pad – Control
- 10. Incontinence Pad - Treated

2. Significance and Use:

This test method is designed to evaluate the resistance of non-leaching antimicrobial treated specimens to the growth of microbes under dynamic contact conditions. This dynamic shake flask test was developed for routine quality control and screening tests in order to overcome difficulties in using classical antimicrobial test methods to evaluate substrate-bound antimicrobials.

This test also allows for the versatility of testing contamination due to such things as hard water, proteins, blood, serum, various chemicals and other contaminants or physical/chemical stresses or manipulations of the specimens of interest. The antimicrobial activity of a substrate bound antimicrobial agent is dependent upon direct contact of microbes with the active chemical agent. This test determines the antimicrobial activity of treated specimens by shaking samples of surface bound materials in a concentrated bacterial suspension for a time period of 10 minutes to 24 hours as specified by the supplier of the samples.

- 2.1 Surface antimicrobial activity is determined by comparing results from the test samples to simultaneously run controls.
- 2.2 The presence of a leaching antimicrobial is both pre and post-determined by the presence of a zone of inhibition. ViaClean Technologies, LLC chose to eliminate the leaching test and washing test.
- 2.3 Stresses may include laundry, wear and abrasion, radiation and steam sterilization, UV exposure, solvent manipulation, temperature susceptibility or similar physical or chemical manipulation.

3. Preparation of Bacterial Inoculum.

Staphylococcus aureus Strain (ATCC #6538) was grown on a Brain Heart Infusion Agar slant for 24 hours and collected in sterile Phosphate Buffer. 50 mL of the diluted culture in Phosphate Buffer was added to each of seven 125 mL flasks.

4. Preparation of the Test Specimen:

A 2 inch by 2 inch square was cut from each fabric. Latex gloves were worn while handling the fabrics.

5. Testing Procedure:

The six samples were each placed into separate 125 mL flasks each containing a 50 mL dilute suspension of *Staphylococcus aureus* in Phosphate Buffer. Each flask was capped with aluminum foil and placed on the shaker. An initial “zero” contact time was established by plating out the control, flask #11, “inoculum only” diluted culture of *Staphylococcus aureus* on 3M APC Petrifilm and incubating for 48 hours at 35° C. A 1 mL aliquot was taken from each of the seven flasks after 24 hours on the shaker. Dilutions of 10⁻² and 10⁻⁴ were made and plated on Petrifilm for counts of the *Staphylococcus aureus* that remained viable.

6. Evaluation of Results:

<u>Sample ID</u>	<u>cfu/mL Staph. (24 hr.)</u>	<u>% Kill</u>	<u>Antibacterial Activity</u>
3. Bed Sheet – Control	720,000	none	0.00
4. Bed Sheet – Treated	15	99.99%	4.23
7. Microfiber Sheet – Control	960,000	none	0.00
8. Microfiber Sheet – Treated	0	100.00%	5.40
9. Incontinence Pad – Control	0	100.00%	5.40
10. Incontinence Pad – Treated	0	100.00%	5.40
11. Initial Staphylococcus count	250,000		
12. Final Staphylococcus count (after 24 hrs.)	190,000	24.00%	

Calculation of the “Antibacterial Activity”: This is the difference in the logarithm of the viable cell count found on an antimicrobial-treated product and a control product after inoculation with, and incubation of, the bacteria. This is also known as the “Log Kill”

<u>Antibacterial Activity</u>	<u>%Kill compared to control</u>	<u>Comment</u>
<1.5	<96.8	poor
1.5 to 2.0	96.8 to 99.0	borderline
2.0 to 3.0	99.0 to 99.9	good
>3.0	>99.9	excellent

3. Conclusions:

A. The treated fabrics showed an excellent kill rate against *Staphylococcus aureus* following 24 hours on the shaker.

B. The control incontinence pad had an excellent kill rate against *Staphylococcus aureus* following 24 hours on the shaker.