Evaluation of TioxoClean for the Inactivation of Feline Calicivirus

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INTRODUCTION

In this report, we present the results on the evaluation of a thin mineral film "coating" based on Ultra Violet PhotoCatalytic (UV-PCO) technology to inactivate bacetra and viruses. Although the core UV-PCO technology was discovered in Japan over 30 years ago, there has been little commercial application of this technology in the US until now. TioxoClean Inc., a New York-based technology development company, has secured critical licenses from the relevant Japanese entities to manufacture a form of UV-PCO technology and is in the process of introducing UV-PCO technology and the range of its potential applications to the US market.

Previous independent tests have established the efficacy of a TioxoClean-coated surface in killing *Eschrichia coli* and S*taphylococcus aureus* bacteria that come in physical contact with the coated surface when exposed to UV-A light. (UV-A light is the near visible range of UV light, commonly known as black light: 320 to 400 nanometers in wave length). Direct sunlight, ambient sunlight (coming through a window, for instance) and fluorescent lighting all provide sufficient UV-A light to trigger the photocatalytic benefits of TioxoClean's technology. Despite the significant difference in the cell walls of bacteria compared to viruses, TioxoClean felt there was reason to assume its UV-PCO coating would prove effective in destroying viruses as well.

A series of tests was conducted to evaluate the efficiency of TioxoClean coatings. The organisms tested were a bacteria (*E. coli*) and virus (feline calicivirus). Feline calicivirus was used as a surrogate of human norovirus (NoV) because NoV cannot be grown routinely in cell cultures while feline calicivirus (FCV) can be grown and titrated easily in cell cultures in the laboratory. Both NoV and FCV belong to the same virus family (Caliciviridae) and have very similar physical and chemical characteristics. In addition, FCV is not harmful to humans although it makes cats sick. The testing protocol adapted for these tests and the results of these tests are attached.

CONCLUSION

In each of the attached tests, the organism exposed to the TioxoClean-coated substrate exhibited an approximate 4 log reduction within one hour of exposure. In layman's terms, this means that 99.99% of the respective organisms were destroyed within one hour of their coming into contact with the UV-A-exposed, TioxoClean-coated stainless steel discs.

Experiment A

Purpose: To determine the anti-FCV performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the *duration of the experiment.*

(To avoid the inhibitory action of salts in the media on TiO2, virus suspension was diluted 1:100 in distilled water before being applied to test coupons)

Method:

1. Expose all test coupons (TioxoClean treated and untreated control) to UV-A (15 watt, 380 nm) for 24 hrs to charge TioxoClean.
2. Apply **DILUTED FCV (1:100 dilution in distilled water; because presence of salt in the virus/cell culture media may inhibit the activity of TiO2)** suspension to control and test coupons (20 µL of virus suspension/coupon). *Continue to expose the test coupons to UV-A light source for the duration of the test period.*
3. After a specific contact time, flood 3 test coupons and 3 control coupons with the eluent (tryptose phosphate broth) using 980 µL of eluent per coupon.
4. Remove the eluate immediately, make serial 10-fold dilutions in MM, and inoculate into CRFK cells grown in 96-well microtiter plates using 4 wells per dilution.
5. Incubate inoculated cells at 37C and examine microscopically for the appearance of cytopathic effects (CPE) after 4 days of incubation.
6. Based on the appearance of CPE, determine the end points and calculate virus titers (amount of virus present).
7. Difference between the amount of virus recovered from control coupon versus that from test coupon will indicate the amount of virus killed by TioxoClean at the indicated time interval.
8. Calculate the amount of virus killed by averaging virus kill in three replicate sets of coupons.
9. Results are shown in Table 9 (page 3).

Table A: Anti-FCV performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the duration of the experiment.

Disc type	Log_{10} virus titer ($TCID_{50}$) after indicated time of exposure															
	0 hr				1 hr				4 hr				24 hr			
	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean
Coated	4.00	4.25	4.75	**4.33**	1.50	0.00	0.00	**0.50**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
Uncoated	4.25	4.25	4.75	**4.41**	2.25	3.25	3.25	**2.91**	1.00	1.25	0.75	**1.00**	0.00	0.00	0.00	**0.00**

Conclusion: After 1 hr exposure, there was ~4 log reduction in virus titer on TiO2 coated discs and only ~1.5 log reduction on untreated discs.

Expt. No B; Date 08 July 07 *(Repetition of Expt. No. 9)* - Anti-FCV performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the *duration of the experiment.*

(To avoid the inhibitory action of salts in the media on TiO2, virus suspension was diluted 1:100 in distilled water and applied on stainless steel discs)

1. Expose all test coupons (TioxoClean treated and untreated control) to UV-A (15 watt, 380 nm) for 24 hrs to charge TioxoClean.
2. Apply **DILUTED FCV (1:100 dilution in distilled water; because presence of salt in the virus/cell culture media could inhibit the activity of TiO2)** suspension to control and test coupons (20 µL of virus suspension/coupon). *Continue to expose the test coupons to UV-A light source for the duration of the test period.*
3. After a specific contact time, flood 3 test coupons and 3 control coupons with the eluent (tryptose phosphate broth) using 980 µL of eluent per coupon.
4. Remove the eluate immediately, make serial 10-fold dilutions in MM, and inoculate in to CRFK cells grown in 96-well microtiter plates using 4 wells per dilution.
5. Incubate inoculated cells at 37C and examine microscopically for the appearance of cytopathic effects (CPE) after 4 days of incubation.
6. Based on the appearance of CPE, determine the end points and calculate virus titers (amount of virus present).
7. Difference between the amounts of virus recovered from control coupon versus that from test coupon will indicate the amount of virus killed by TioxoClean at the indicated time interval.
8. Calculate the amount of virus killed by averaging virus kill in three replicate sets of coupons.

Table B: Anti-FCV performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the duration of the experiment.

Disc type	Log_{10} median virus titer ($TCID_{50}$) after indicated exposure time															
	0 hr				1 hr				4 hr				24 hr			
	Disc 1	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**
Coated	4.50	4.25	4.25	**4.33**	0.75	0.75	0.00	**0.50**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
Uncoated	4.25	5.00	4.50	**4.58**	3.75	3.50	3.75	**3.66**	0.75	0.75	0.00	**0.50**	0.00	0.00	0.00	**0.00**

Conclusion: After 1 hr exposure, there was ~4 log reduction in virus titer on TiO2 coated discs and only ~1.0 log reduction on untreated discs.

Expt. No C; Date 07 July 07: Anti-*E coli* performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the *duration of the experiment.*

(To avoid the inhibitory action of salts in the media on TiO2, bacterial suspension was diluted 1:100 in distilled water and applied on stainless steel discs)

1. Expose all test coupons (TioxoClean treated and untreated control) to UV-A (15 watt, 380 nm) for 24 hrs to charge TioxoClean.

2. Apply **DILUTED *E coli* (1:100 dilution in distilled water; because presence of organic substances in the bacterial culture media may inhibit the activity of TiO2)** suspension to control and test coupons (20 µL of bacterial suspension/coupon). *Continue to expose the test coupons to UV-A light source for the duration of the test period.*

3. After a specific contact time, flood 3 test coupons and 3 control coupons with the eluent (tryptose phosphate broth) using 980 µL of eluent per coupon.

4. Remove the eluate immediately, make serial 10-fold dilutions in PBS, and inoculate TSA plates by spread plate method.

5. Incubate inoculated plates at 37C for 24 hrs and count bacterial colonies.

6. Calculate the numbers of viable bacteria.

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Table C: Anti-*E coli* performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the duration of the experiment.

Disc type	Recovery of viable bacteria (Log_{10} CFU) after indicated exposure time															
	0 hr				1 hr				4 hr				24 hr			
	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean
Coated	4.11	4.60	4.25	**4.32**	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
Uncoated	4.07	4.11	4.34	**4.17**	4.47	4.46	4.32	**4.41**	0	0	0	**0**	0	0	0	**0**

Conclusion: After 1 hr exposure, there was ~4 log reduction in bacterial numbers on TiO2 coated discs and no bactericidal effect could be observed in untreated discs at the same exposure time. However, after 4 hr exposure, no viable bacteria could be recovered from both treated and untreated discs.

Expt. No D; Date 10 July 07 (repletion of Expt. No 11 but with 2 additional exposure times)

Anti-*E coli* performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the *duration of the experiment.*

1. Expose all test coupons (TioxoClean treated and untreated control) to UV-A (15 watt, 380 nm) for 24 hrs to charge TioxoClean.

2. Apply **DILUTED *E coli* (1:100 dilution in distilled water; because presence of organic substances in the bacterial culture media may inhibit the activity of TiO2)** suspension to control and test coupons (20 µL of bacterial suspension/coupon). *Continue to expose the test coupons to UV-A light source for the duration of the test period.*

3. After a specific contact time, flood 3 test coupons and 3 control coupons with the eluent (tryptose phosphate broth) using 980 µL of eluent per coupon.

4. Remove the eluate immediately, make serial 10-fold dilutions in PBS, and inoculate in to TSA plates by spread plate method.

5. Incubate inoculated plates at 37C for 24 hrs and count the bacterial colony.

6. Calculate bacterial numbers.

Table D: Anti-*E coli* performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the duration of the experiment.

Disc type	Recovery of viable bacteria (Log_{10} CFU) after indicated exposure time																			
	0 hr				0.5 hr				1hr				2 hr				4 hr			
	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mean	Disc 1	Disc 2	Disc 3	Mea
Coated	4.99	4.60	4.96	**4.85**	4.85	4.77	4.77	**4.72**	0	0	0	**0**	0	0	0	**0**	0	0	0	**0**
Uncoated	4.74	4.83	4.73	**4.76**	4.60	4.95	4.79	**4.78**	4.79	4.90	4.51	**4.73**	0	0	0	**0**	0	0	0	**0**

Conclusion: After 1 hr exposure, there was ~4 log reduction in bacterial numbers on TiO2 coated discs and no bactericidal effect could be observed in untreated discs at the same exposure time. However, after 2 hr exposure, no viable bacteria could be recovered from both treated and untreated discs.

Expt. No E; Date 13 July 07 (Repetition of Expt. No. 9 and 10 with two additional exposure times)

Anti-FCV performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the *duration of the experiment.*

1. Expose all test coupons (TioxoClean treated and untreated control) to UV-A (15 watt, 380 nm) for 24 hrs to charge TioxoClean.

2. Apply **DILUTED FCV (1:100 dilution in distilled water; because presence of salt in the virus/cell culture media could inhibit the activity of TiO2)** suspension to control and test coupons (20 µL of virus suspension/coupon). *Continue to expose the test coupons to UV-A light source for the duration of the test period.*

3. After a specific contact time, flood 3 test coupons and 3 control coupons with the eluent (tryptose phosphate broth) using 980 µL of eluent per coupon.

4. Remove the eluate immediately, make serial 10-fold dilutions in MM, and inoculate in to CRFK cells grown in 96-well microtiter plates using 4 wells per dilution.

5. Incubate inoculated cells at 37C and examine microscopically for the appearance of cytopathic effects (CPE) after 4 days of incubation.

6. Based on the appearance of CPE, determine the end points and calculate virus titers (amount of virus present).

7. Difference between the amounts of virus recovered from control coupon versus that from test coupon will indicate the amount of virus killed by TioxoClean at the indicated time interval.

8. Calculate the amount of virus killed by averaging virus kill in three replicate sets of coupons.

Table E: Anti-FCV performance of TiO2 coated stainless steel discs, charged with UV-A light for 24 hrs, and then continuously exposed to UV-A for the duration of the experiment.

Disc type	Log_{10} median virus titer ($TCID_{50}$) after indicated exposure time																			
	0 hr				0.5 hr				1hr				2 hr				4 hr			
	Disc 1	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**	**Disc 1**	**Disc 2**	**Disc 3**	**Mean**
Coated	4.25	4.00	4.00	**4.08**	3.25	3.00	2.50	**2.91**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
Uncoated	3.75	4.50	3.75	**4.00**	4.25	4.25	4.00	**4.08**	3.25	3.75	3.25	**3.41**	0.75	1.25	1.75	**1.25**	0.00	1.00	0.00	**0.33**

Conclusion: After 1 hr exposure, there was ~4 log reduction in virus titer on TiO2 coated discs and only ~1.0 log reduction on untreated discs.