MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antibacterial Activity and Sanitizing Efficacy of Florman Orthodontics' Test Substance

Test Method

Modified ASTM International Method E1153
Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces

Study Identification Number

NG6727

Study Sponsor

Eversmile, Inc.
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Test Facility

Microchem Laboratory
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ASTM E1153: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. ASTM E1153 is a quantitative test method designed to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned inanimate, nonporous, non-food contact surfaces. The method is typically used with a maximum contact time of 5 minutes, during which the sanitizer reduces the concentration of viable test microorganisms. ASTM E1153 utilizes non-antimicrobial agents as controls to establish baselines for microbial reductions. The ASTM E1153 method is a benchmark method for non-food contact surface sanitizers and is recognized by several regulatory agencies as an approved method for claim substantiation.

Laboratory Qualifications Specific to ASTM E1153

Microchem Laboratory began conducting the ASTM E1153 test method in 2007. Since then, the laboratory has performed hundreds of ASTM E1153 tests on a broad array of test substances, against a myriad of bacterial and fungal species. The laboratory is also experienced with regard to modifying the test method as needed in order to accommodate customer needs. Every ASTM E1153 test at Microchem Laboratory is performed in a manner appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the method.

Study Timeline

Culture Initiated	Surfaces Inoculated	Surfaces Treated	Surfaces Evaluated	Report Delivered
S. aureus* & *E. coli				
08FEB2016	09FEB2016	09FEB2016	10FEB2016	10MAR2016
S. mutans				
09FEB2016	11FEB2016	11FEB2016	15FEB2016	10MAR2016
S. pneumoniae				
22FEB2016	24FEB2016	24FEB2016	26FEB2016	10MAR2016

Test Substance Information

The test substance was received on 08 February 2016 and the following pictures were taken.
(note: photos depict the test substance that was analyzed in this study)

Test Substances Received: Eversmile

Test Substances arrived ready to use. Test substances were not diluted prior to use in the study.

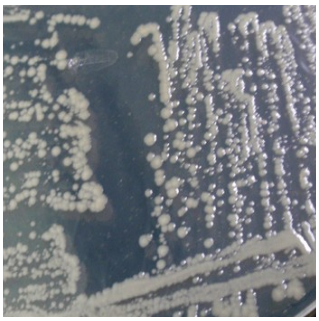
Test Microorganism Information

The test microorganism(s) selected for this test:

Staphylococcus aureus 6538

This bacterium is a Gram-positive, spherical-shaped, facultative anaerobe. *Staphylococcus* species are known to demonstrate resistance to antibiotics such as methicillin. *S. aureus* pathogenicity can range from commensal skin colonization to more severe diseases such as pneumonia and toxic shock syndrome (TSS). *S. aureus* is commonly used in several test methods as a model for gram positive bacteria. It can be difficult to disinfect but does demonstrate susceptibility to low level disinfectants.

Test Microorganism Information



Escherichia coli **8739**

This bacteria is a Gram-negative, rod shaped, facultative anaerobe commonly found in the gastrointestinal tract of mammals. Although most serotypes of this microorganism are harmless there are pathogenic groups of *E. coli* such as enterohemorrhagic (EHEC), verocytotoxin producing (VTEC) and Shiga-like toxin producing (STEC) that can cause a multitude of illnesses. *E. coli* is relatively susceptible to disinfection when dried on a surface, yet it can be a challenging microorganism to mitigate in solution.

Streptococcus pneumoniae **49619**

This bacteria is Gram-positive, aerotolerant, and spherical-shaped. S. pneumonia is part of the normally occurring flora of the upper respiratory tract and can be responsible for numerous infections including pneumonia, bacteremia, and meningitis. *S. pneumoniae* has exhibited susceptibility to several antimicrobial products and is moderately easy to disinfect.

Streptococcus mutans **25175**

Streptococcus mutans is a Gram-positive, facultatively anaerobic cocci. This microorganism is commonly found in the human oral cavity and contributes to tooth decay. To survive in the oral cavity, *S. mutans* must be able to survive frequent and rapid environmental changes. *S. mutans* is closely related to *S. sobrinus* and the two organisms often co-habitate.

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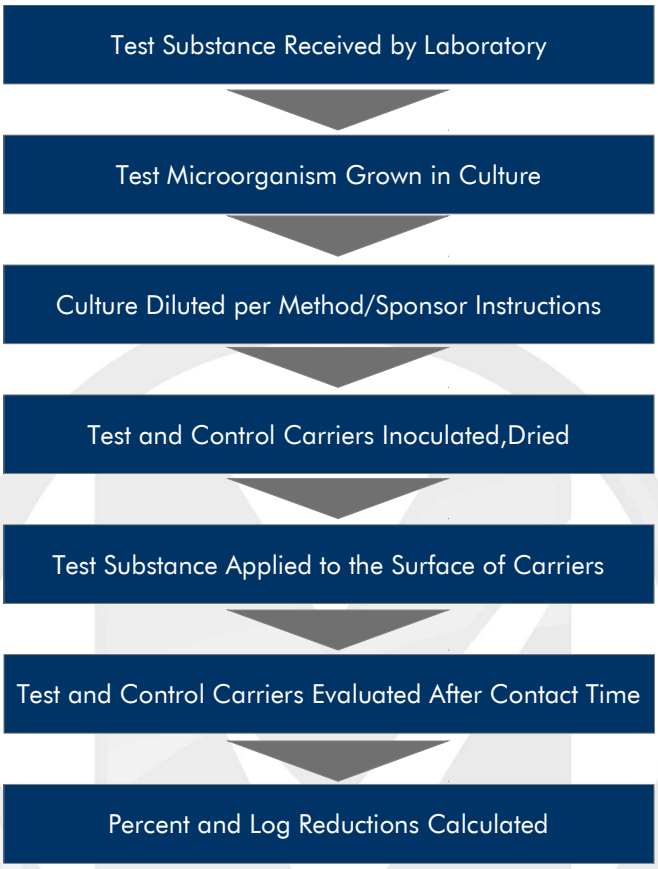
Diagram of the Procedure



Summary of the Procedure

- The test microorganism is prepared, usually by growth in liquid culture medium.
- The test culture may be supplemented with an artificial soil load, such as horse or fetal bovine serum, for one-step cleaner/sanitizer claims.
- Sterilized carriers are inoculated with a volume of the test culture. Inoculated slides are dried in an incubator. Only completely dried carriers are used in the test.
- Test carriers are treated with the test substance and incubated for the predetermined contact time.
- Control carriers are treated with a buffered saline solution and are allowed to sit for the predetermined contact time.
- At the conclusion of the contact time, test and control carriers are chemically neutralized.
- Dilutions of the neutralized test substance are evaluated using appropriate growth media to determine the surviving microorganisms at the respective contact time.
- The effect of the test substance is compared to the effect of the control substance in order to determine microbial reductions.

Criteria for Scientific Defensibility of an ASTM E1153 Study

For Microchem Laboratory to consider an ASTM E1153 study to be scientifically defensible, the following criteria must be met:

1. The average number of viable microorganisms recovered from the control carriers must be approximately 7.5 x 10^5 cells/carrier or greater.
2. Ordinary consistency between replicates must be observed for the control carriers.
3. Positive/Growth controls must demonstrate growth of appropriate test microorganism.
4. Negative/Purity controls must demonstrate no growth of test microorganism.

Passing Criteria

ASTM International defines passing criteria to be a 3 Log_{10} or 99.9% reduction in the treated test carriers when compared to the control carriers.

Testing Parameters used in this Study

Test Substance Diluent:	N/A (Prepared on-site)	Carrier Type:	18 x 36 mm glass slides
Carriers per Test:	1 Test, 1 Control	Volume Test Substance:	5.0 ml

Parameters for *S. aureus* and *E. coli*

Culture Growth Media:	Tryptic Soy Broth	Culture Transfer Number:	1
Culture Growth Time:	24-48 hours	Culture Supplement:	N/A
Carrier Inoculum Volume:	0.020 ml	Carrier Inoculation Area:	1 $inch^2$
Inoculation Concentration:	>1 x 10^6 CFU/Carrier	Carrier Dry Time:	17 minutes
Carrier Dry Temperature:	36.0°C		
Contact Time:	30 seconds	Neutralization Media:	D/E w/1% Catalase
Plate Incubation Time:	24-48 hours	Plate Incubation Temp:	36.0°C

Parameters for *S. mutans* and *S. pneumoniae*

Culture Growth Media:	Tryptic Soy Agar w/blood	Culture Transfer Number:	1
Culture Growth Time:	24-48 hours	Culture Supplement:	N/A
Carrier Inoculum Volume:	0.020 ml	Carrier Inoculation Area:	1 $inch^2$
Inoculation Concentration:	>1 x 10^6 CFU/Carrier	Carrier Dry Time:	20-30 minutes
Carrier Dry Temperature:	36.0°C		
Contact Time:	60 seconds	Neutralization Media:	D/E w/1% Catalase
Plate Incubation Time:	24-48 hours	Plate Incubation Temp:	36±1°C + 5% CO_2

Study Photographs

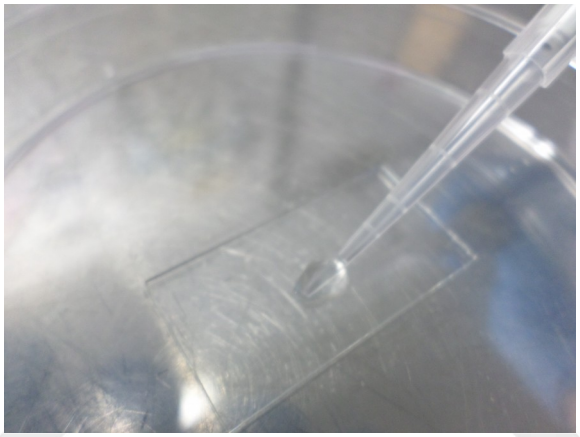


Photo: Carrier inoculation

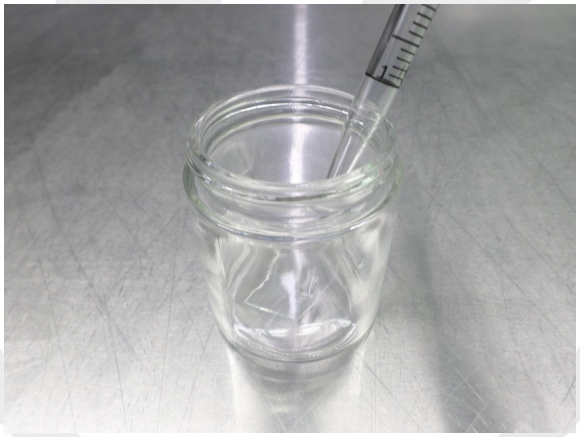


Photo: Carrier treatment

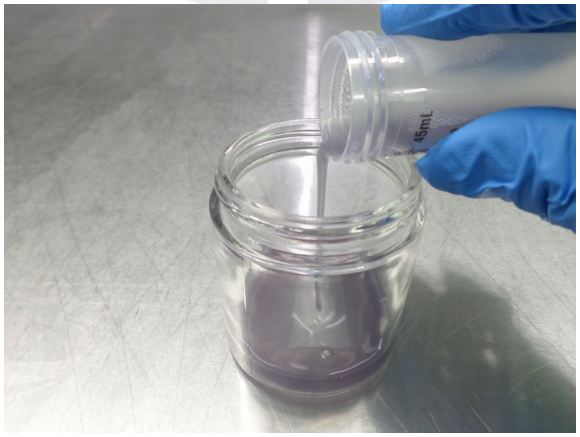


Photo: Carrier neutralization

Control Results

Neutralization Method: Confirmed
Growth Confirmation: Confirmed
Media Sterility: Confirmed

Calculations

$$\text{Percent Reduction} = \left(\frac{B - A}{B}\right) \times 100$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

$$\text{Log}_{10}\text{Reduction} = \text{Log}\left(\frac{B}{A}\right)$$

Where:
B = Number of viable test microorganisms on the control carriers after the contact time
A = Number of viable test microorganisms on the test carriers after the contact time

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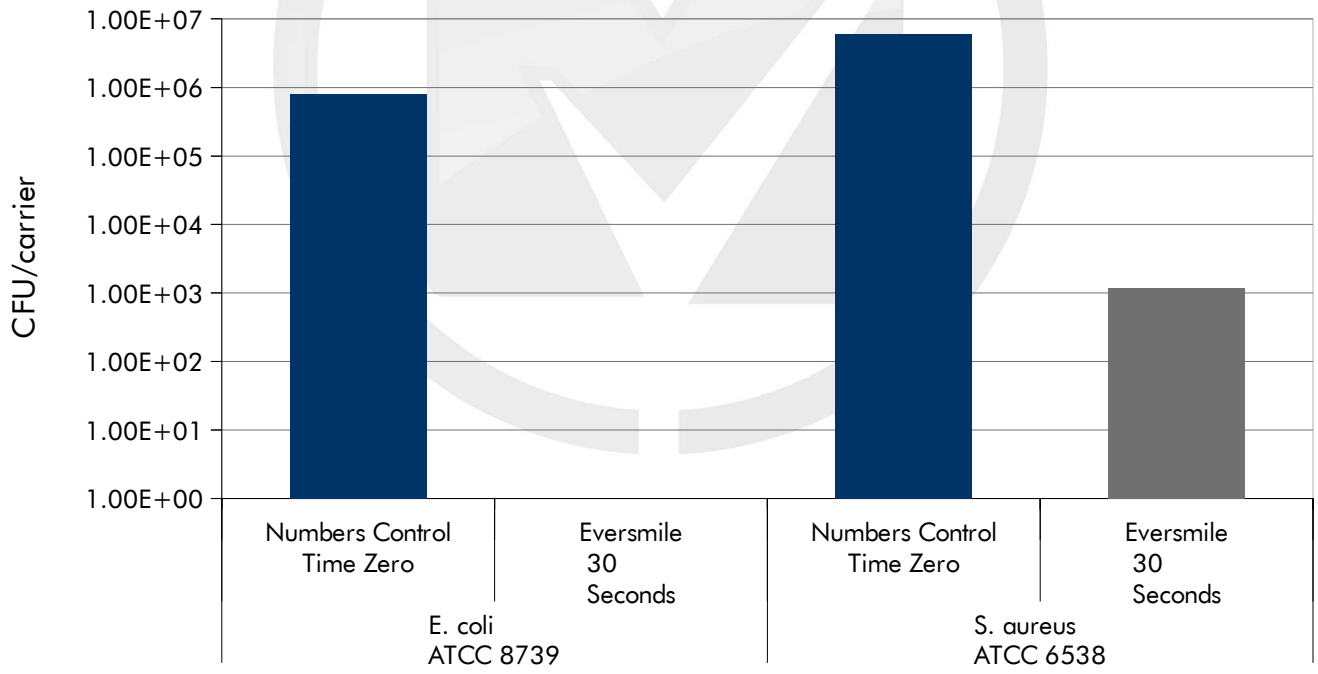
Results of the Study

Test Microorganism	Contact Time	Test Substance	CFU/carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
E. coli ATCC 8739	Time Zero	Numbers Control	8.13E+05	N/A	
	30 Seconds	Eversmile	<1.25E+01	>99.998%	>4.81
S. aureus ATCC 6538	Time Zero	Numbers Control	6.00E+06	N/A	
	30 Seconds	Eversmile	1.19E+03	99.98%	3.70

**The limit of detection for this assay is 1.25E+01.*



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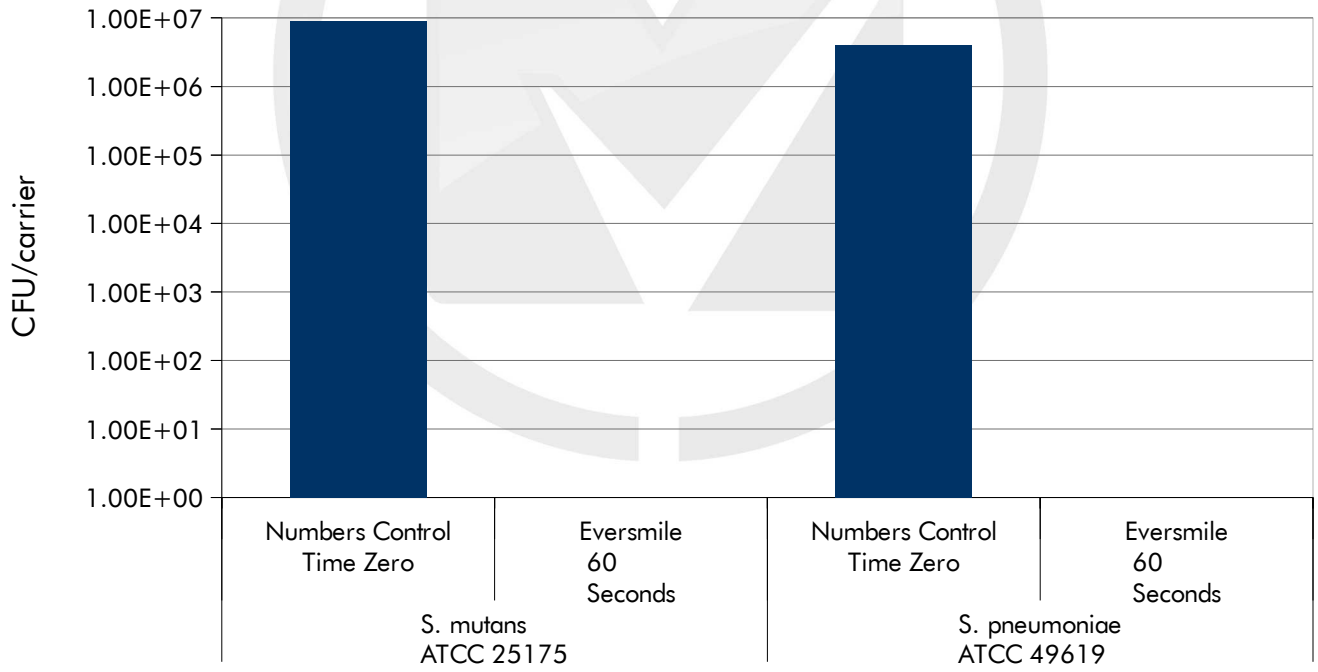
Results of the Study

Test Microorganism	Contact Time	Test Substance	CFU/carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
S. mutans ATCC 25175	Time Zero	Numbers Control	9.00E+06	N/A	
	60 Seconds	Eversmile	<1.25E+01	>99.99986%	>5.86
S. pneumoniae ATCC 49619	Time Zero	Numbers Control	4.00E+06	N/A	
	60 Seconds	Eversmile	<1.25E+01	>99.9997%	>5.51

**The limit of detection for this assay is 1.25E+01*



RESULTS

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The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

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