FINAL REPORT

Standard Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Material

PROTOCOL: ASTM E 2180

ORDER NO: 030735622

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Certificate of Analysis

Client: Casco Bay Molding, LTD

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Project: ASTM E2180—Standard Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Material

Product: Silicone Mouthpieces

EMSL NO: 030735622

Sample received: 10/19/2007

Start date: 11/01/2007

Completion Date: 11/05/2007

Experimental Summary:

Two samples were received by the laboratory for testing: one non-antimicrobial silicone mouthpiece and one test silicone mouthpiece (80/20; 3.5%). The ASTM method E2180 was performed, which is the method used "to evaluate (quantitatively) the effectiveness of agents incorporated or bound into or onto mainly flat (two dimensional) hydrophobic or polymeric surfaces" (Test Method E2180, pg1). The test organisms utilized were *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442. Two separate agar slurries were prepared, one for each organism. The agar slurries contained 1 mL of the test organism (concentration= 7.0×10^8 cells/mL), 0.85 g NaCl, 0.3 g agar, and 100 mL of deionized water. The final inoculum concentration was equal to ~7.0×10^6 cells/mL.

The control sample was prepared in triplicate and aseptically cut into twelve equally sized pieces. One mL of each agar slurry was applied to the prepared samples. Using both sonication and manual vortexing the agar slurry was immediately removed from one set of the control samples and plate counts were performed. The data recovered was designated '0 hours'.

The remaining set of control samples and the treated material were incubated at 35°C for 24 hours with the solidified agar slurry intact. The agar slurry was again removed and processed with sonication and vortexing. Plate counts were performed. The data retrieved from this set of samples was designated '24 hours'.

Calculation of 'percent reduction' compares the geometric mean of each time point data set with that of the relevant time point control.

Experimental Results:

Table 1.1—Colony forming units (CFU) collected after control and test mouthpieces were exposed to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Colony forming units are based on the average of three plate counts.

Sample Identification		**Contact Time '0 Hours'**		**Contact Time '24 Hours'**		**Percent Reduction**	
		P. aeruginosa CFU/mL	*S. aureus* CFU/ mL	*P. aeruginosa* CFU/ mL	*S. aureus* CFU/ mL	*P. aeruginosa*	*S. aureus*
Control	Avg	4,080,000	3,700,000	86,300,000	66,000,000		
	GM	1,860,000	3,230,000	81,200,000	54,900,000		
Sample 1 (80/20)	Avg			10,500,000	67,600	91.2%	99.9%
	GM			7,070,000	53,700		

Avg: Average of the three triplicate values
GM: Geometric Mean of the three triplicate values (used to calculate Percent Reduction)

Table 1.2—Raw data for triplicate counts for both samples, at 2 time points, inoculated with *Pseudomonas aeruginosa* and *Staphylococcus aureus*. (CFU/mL)

Sample Identification	Contact time '0 Hours'		Contact Time '24 Hours'	
	P. aeruginosa **CFU/ mL**	***S. aureus*** **CFU/ mL**	***P. aeruginosa*** **CFU/ mL**	***S. aureus*** **CFU/ mL**
Control	930,000	6,400,000	123,000,000	92,000,000
	10,600,000	2,300,000	74,000,000	83,000,000
	720,000	2,400,000	62,000,000	23,000,000
Sample 1 (80/20)			7,200,000	98,000
			22,000,000	19,000
			2,400,000	86,000