MONASH University

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GENOMICS, BIOTECHNOLOGY AND DIAGNOSTICS

Mr Robert White
SDI International Pty Ltd
33-37 Villas Road
Dandenong South, Victoria, 3175

15th of August 2011

Dear Robert,

We are writing to inform you that testing of the sample you submitted is complete. You requested that we determine the antimicrobial properties of a potentially antimicrobial compound, using the JIS methodology JIS Z 2801:2000(E).

The sample received from you was **Access Multipurpose Cleaner**. You also provided three test pieces and six control pieces (**laminate**) 50 mm x 50 mm that were used for analysis.

According to the standard, each control and test piece for all three samples were cleaned by wiping lightly with 80% ethanol and then placed in individual sterile Petri dishes. Access Multipurpose Cleaner was then applied to three test pieces according to the manufacturer's instructions listed on the bottle. For analysis of the immediate effect of the Access Multipurpose Cleaner, each test piece and six control pieces were then inoculated with 0.4 ml of a culture of *Staphylococcus aureus* ATCC6538 that had been adjusted by dilution to approximately 2.5×10^5 cells per ml.

For analysis of the effect of the Access Multipurpose Cleaner 24 hours after application, Access Multipurpose Cleaner was applied to three test pieces according to the manufacturer's instructions listed on the bottle and then allowed to rest for 24 hours. Each test piece and six control pieces were then inoculated with 0.4 ml of a culture of *Staphylococcus aureus* ATCC6538 that had been adjusted by dilution to approximately 2.5×10^5 cells per ml.

The inoculum was covered with a film measuring 40 x 40 mm and the film pressed to spread the inoculum over the entire surface area of the sample covered by the film. The lid was then placed on the Petri dish. The Petri dishes containing three control pieces and three test pieces for each sample were then incubated at 35°C (relative humidity of approximately 90%) for 24 hours. The three remaining Petri dishes containing control pieces from each sample were processed immediately to determine the base line viable count.

To test the viable number of bacterial cells present from each of the control pieces, both prior to and following incubation, and the test pieces, 10 ml of SCDLP broth was added to the Petri dishes containing the pieces, and the Petri dish was then shaken for 10 minutes on an orbital shaker. Following this, 1 ml of the washings was taken from each test and control piece and diluted in sterile physiological saline. One ml aliquots of various dilutions were added to duplicate 15 ml of molten plate count agar and mixed thoroughly. The plate count agar was then poured into sterile Petri dishes and allowed to set. Plates were then incubated at 35°C (relative humidity of approximately 90%) for 40 hours. Following incubation the number of colonies present on each plate was recorded and a viable count calculated.

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The results recorded from these plates are given in the tables below:

Table 1: Viable Counts for samples when *S. aureus* was used as an inoculum

	Viable Count (cfu/ml)	
	Access Multipurpose Cleaner T_0	**Access Multipurpose Cleaner T_{24}**
Prior to Incubation:		
Control 1	1.41×10^7	4.10×10^6
Control 2	1.52×10^7	2.65×10^6
Control 3	1.46×10^7	4.50×10^6
Post Incubation:		
Control 4	1.14×10^6	5.00×10^2
Control 5	3.02×10^6	1.17×10^3
Control 6	1.50×10^6	5.65×10^5
Post Incubation:		
Test 1	<10	<10
Test 2	<10	<10
Test 3	55	<10

We then took an average of the viable counts for the three controls prior to incubation, the three controls post incubation, and the three test pieces for each of the samples.

This data is presented in the tables below:

Table 2: Average viable counts for pre and post incubation controls and test pieces when *S. aureus* was used as an inoculum.

	Average Viable Count (cfu/ml)	
	Access Multipurpose Cleaner T_0	**Access Multipurpose Cleaner T_{24}**
Prior to Incubation Untreated Controls	1.46×10^6	3.75×10^6
Post Incubation: Untreated Controls	1.89×10^6	1.89×10^5
Post Incubation: Treated Test Pieces	25	10

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The efficiency of each of the tests was determined using the following formula based on the results reported in the above tables:

$(L_{max}- L_{min})/(L_{mean}) \leq 0.2$

Where:

L_{max} : maximum logarithm of the number of viable cells of bacteria immediately following inoculation on untreated test pieces.
L_{min} : minimum logarithm of the number of viable cells of bacteria immediately following inoculation on untreated test pieces.
L_{mean} : average logarithm of the number of viable cells of bacteria immediately following inoculation on untreated test pieces.

The test was judged as being effective when the above equation was satisfied.

Logarithmic values of the number of viable cells of bacteria immediately following inoculation on the untreated test pieces are reported in the tables below:

Table 3: Logarithms for untreated samples following inoculation when *S. aureus* was used as an inoculum

	Logarithmic value of viable cells	
	Access Multipurpose Cleaner T_0	**Access Multipurpose Cleaner T_{24}**
Prior to Incubation:		
Control 1	7.15 **(L_{min})**	6.61
Control 2	7.18**(L_{max})**	6.42**(L_{min})**
Control 3	7.16	6.65**(L_{max})**
L_{mean}	7.16	6.56
$(L_{max}- L_{min})/(L_{mean})$	0.003	0.035

Based on the above data all tests were determined to be effective as the equation was satisfied in each instance.

The value of the antimicrobial activity was then calculated for each test using the following equation:

R = [log(B/A)-log(C/A)]=[log (B/C)]

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Where:

R : value of antimicrobial activity
A : average of the number of viable cells of bacteria immediately after inoculation on the untreated test pieces
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 treated test piece after 24 hours

Higher numbers for the value of R indicate better antimicrobial activity.

The values of R, A, B and C are recorded in the following tables:

Table 4: **Values of R, A, B and C when *S. aureus* was used as an inoculum.**

	Average Viable Count (cfu/ml)	
	Access Multipurpose Cleaner T_0	**Access Multipurpose Cleaner T_{24}**
Prior to Incubation Untreated Controls **[A]**	1.46×10^6	3.75×10^6
Post Incubation: Untreated Controls **[B]**	1.89×10^6	1.89×10^5
Post Incubation: Treated Test Pieces **[C]**	25	10
Antimicrobial Activity [R]	4.9	4.3
% Reduction	>99.99%	>99.99%

Comments:

The tests conducted were deemed to be effective as dictated by standard JIS Z 2801:2000(E).

As is indicated by the positive values for Antimicrobial Activity [R] the product, Access Multipurpose Cleaner, has significant antimicrobial activity against *Staphylococcus aureus* when tested on laminate. This level of activity (>3 for Antimicrobial Activity, and >99.9% for the % Reduction) is categorized as strong activity. Further, this level of activity was retained even when the product had been applied 24 hours previous to the challenge with the bacteria.

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We hope that this information included in this report assists you in the effective running of your service. If you require any further information regarding the outcomes of these tests or the methodology and justifications used in the testing procedure, please feel free to contact us at any time.

Yours sincerely,

Dr Kylie Wilson (Senior Technical Officer)

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