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Dear Robert,

We are writing to inform you that testing of the samples 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 **RIDOV**. You also provided three test pieces and six control pieces (**laminite**) 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. RIDOV was then applied to three test pieces according to the manufacturer's instructions listed on the bottle. For analysis of the immediate effect of RIDOV, each test piece and six control pieces were then inoculated with 0.4 ml of a culture of Vancomycin-Resistant Enterococci (**VRE**) that had been adjusted by dilution to approximately 2.5×10^5 cells per ml.

For analysis of the effect of RIDOV 72 hours after application, RIDOV was applied to three test pieces according to the manufacturer's instructions listed on the bottle and then allowed to rest for 72 hours. Each test piece and six control pieces were then inoculated with 0.4 ml of a culture of Multiply Resistant Vancomycin-Resistant Enterococci (**VRE**) 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

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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.

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

Table 1: Viable Counts for samples when VRE was used as an inoculum.

	Viable Count (cfu/ml)	
	RIDOV T ₀	RIDOV T ₇₂
Prior to Incubation:		
Control 1	2.36 x 10 ⁷	1.46 x 10 ⁷
Control 2	1.82 x 10 ⁷	1.64 x 10 ⁷
Control 3	1.63 x 10 ⁷	2.34 x 10 ⁷
Post Incubation:		
Control 4	1.22 x 10 ⁷	3.15 x 10 ⁶
Control 5	1.82 x 10 ⁷	2.50 x 10 ⁵
Control 6	2.46 x 10 ⁷	4.50 x 10 ⁵
Post Incubation:		
Test 1	19.5	<10
Test 2	<10	<10
Test 3	<10	<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 VRE was used as an inoculum.

	Average Viable Count (cfu/ml)	
	RIDOV T ₀	RIDOV T ₇₂
Prior to Incubation Untreated Controls	1.94 x 10 ⁷	1.81 x 10 ⁷
Post Incubation: Untreated Controls	1.83 x 10 ⁷	1.28 x 10 ⁶
Post Incubation: Treated Test Pieces	13.17	10

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

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

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 VRE was used as an inoculum.

	Logarithmic value of viable cells	
	RIDOV T_0	RIDOV T_{72}
Prior to Incubation:		
Control 1	7.37 (L_{\max})	7.16 (L_{\min})
Control 2	7.26	7.22
Control 3	7.21 (L_{\min})	7.37 (L_{\max})
L_{mean}	7.28	7.25
$(L_{\max} - L_{\min}) / (L_{\text{mean}})$	0.02	0.03

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:

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

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 table:

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

	Average Viable Count (cfu/ml)	
	RIDOV T ₀	RIDOV T ₇₂
Prior to Incubation Untreated Controls [A]	1.94 x 10 ⁷	1.81 x 10 ⁷
Post Incubation: Untreated Controls [B]	1.83 x 10 ⁷	1.28 x 10 ⁶
Post Incubation: Treated Test Pieces [C]	13.17	10
Antimicrobial Activity [R]	6.14	5.11
% 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, RIDOV, has a high level of antimicrobial activity against VRE when tested on laminate. This level of activity (>3.0 for Antimicrobial Activity, and >99.99% for the % Reduction) is categorized as strong activity.

It is worth noting that this strong activity is still evidenced 72 hours after RIDOV has been applied to the test surface, and that the product, when applied to laminate produces a surfactant-like quality, which makes it difficult to retain the bacterial test sample on the surface of the test piece. The activity reported here may in actual fact be even more significant, as it is possible that the bacteria were able to escape killing because they may have “slipped” off the test piece during the 24 incubation step.

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)



Dr. Kunkun Zhang (Technical Officer)