# **TEST REPORT**

Test No. 2019D-BT-224 April 24, 2019

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Test No.2019D-BT-224

## Anti-bacterial test

#### 1. Purpose

The test was conducted to estimate the anti-bacterial activity of the sample.

### 2. Test Sample

JJJee

#### 3. Test bacteria

Legionella pneumophila

ATCC 33512

#### 4. Test Method

### 1) Preparation of the cell suspension

The test bacteria was cultured on buffered charcoal-yeast extract agar with 0.1%  $\alpha$ -ketoglutalate agar (BCYE $\alpha$  agar, bioMérieux Japan Ltd.) for 48-72 hours anaerobically at 37°C. After incubation, the culture was suspended in normal saline solution and the solution contained  $10^{7\sim8}$  cells per 1 mL.

These dilutions were used as the cell suspension for inoculation.

#### 2) Anti-bacterial test procedures

Transfer 0.1 mL cell suspension to 10 mL test solution. After 10 seconds, the 10-fold dilutions of test solutions were prepared using normal saline solution. Then each dilution were dispensed to BCYEα agar. As control, the normal saline solution was used instead of test solution. The results were calculated from the number of colonies of each test plate by appropriate dilution series.

### 5. Test Results

The results of the anti-bacterial activity test of the sample are shown in table 1

Table 1. Results of Anti-bacterial Test of JJJee against Legionella pneumophila

Test sample	Viable cell count (CFU /mL)	
	Initial	After 10 seconds*
JJJee	4.2×10 <sup>6</sup>	<10 <sup>1</sup> (>99.99%
Control	4.2×10 <sup>6</sup>	3.8×10 <sup>6</sup>
Normal saline solution was used a	s control,	
<10 <sup>1</sup> : Below the detection limit, *m	ean value of n=3.	
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