

## TEST REPORT

Test No.28D-BT-265-1

September 9<sup>th</sup>, 2016

Requested by : PH+Cleanee Inc.

Test sample : JJJee

Test item : Antimicrobial test

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## Antimicrobial test

### 1. Purpose

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

### 2. Test Sample

JJee

### 3. Test bacteria and yeast

<i>Escherichia coli</i>	NBRC 3972
<i>Salmonella enterica</i>	NBRC 100797
<i>Pseudomonas aeruginosa</i>	NBRC 13275
<i>Staphylococcus aureus</i>	NBRC 13276
<i>Listeria monocytogenes</i>	ATCC 19111
<i>Candida albicans</i>	NBRC 1594

### 4. Test Method

#### 1) Preparation of the cell suspension

The test bacteria were cultured on nutrient agar (Nissui Co.,Ltd) for 24 hours at 35°C. After incubation, the culture were suspended in normal saline solution and the solution contain  $10^8$  cells per 1mL.

The test yeast was cultured on potato dextrose agar (Nissui Co.,Ltd) for 48 hours at 25°C. After incubation, the culture was suspended in normal saline solution and the solution contain  $10^8$  cells per 1mL.

These dilutions were used as the cell suspension for inoculation.

#### 2) Antimicrobial test procedures

Transfer 0.1 ml of cell suspensions to the 10 mL of each test solutions and incubated at 25°C. After incubations, the 10-fold dilutions of test solutions were prepared using sterilized normal saline solution. And each dilution was dispensed to SCDLP plates or GPLP plates. And also, the normal saline solution was used as

control. The results were calculated from the numbers of colonies of each test plates by appropriate dilution series.

#### 5. Test Results

The results of the Antimicrobial activity test of the samples were shown in table 1~6.

Table 1. Results of Antimicrobial Test of JJJee against *Escherichia coli*.

Test sample	Initial	Viable cell count (cfu /mL)		
		10 seconds	30 seconds	5 minutes
JJJee	4.5×10 <sup>6</sup>	<10 <sup>1</sup> (>99.9%)	<10 <sup>1</sup> (>99.9%)	<10 <sup>1</sup> (>99.9%)
Control	4.5×10 <sup>6</sup>	4.6×10 <sup>6</sup>	4.5×10 <sup>6</sup>	4.5×10 <sup>6</sup>

Normal saline solution was used as control.

Table 2. Results of Antimicrobial Test of JJJee against *Salmonella enterica*.

Test sample	Initial	Viable cell count (cfu /mL)		
		10 seconds	30 seconds	5 minutes
JJJee	6.4×10 <sup>6</sup>	<10 <sup>1</sup> (>99.9%)	<10 <sup>1</sup> (>99.9%)	<10 <sup>1</sup> (>99.9%)
Control	6.4×10 <sup>6</sup>	5.5×10 <sup>6</sup>	5.1×10 <sup>6</sup>	5.3×10 <sup>6</sup>

Normal saline solution was used as control.

Table 3. Results of Antimicrobial Test of JJJee against *Pseudomonas aeruginosa*.

Test sample	Initial	Viable cell count (cfu /mL)		
		10 seconds	30 seconds	5 minutes
JJJee	3.6×10 <sup>6</sup>	<10 <sup>1</sup> (>99.9%)	<10 <sup>1</sup> (>99.9%)	<10 <sup>1</sup> (>99.9%)
Control	3.6×10 <sup>6</sup>	5.5×10 <sup>6</sup>	5.6×10 <sup>6</sup>	3.7×10 <sup>6</sup>

Normal saline solution was used as control.

Table 4. Results of Antimicrobial Test of JJJee against *Staphylococcus aureus*.

Test sample	Initial	Viable cell count (cfu /mL)	
		30 seconds	30 minutes
JJJee	$2.9 \times 10^6$	$1.4 \times 10^6$ (44.0%)	$4.7 \times 10^2$ (>99.9%)
Control	$2.9 \times 10^6$	$2.5 \times 10^6$	$2.6 \times 10^6$

Normal saline solution was used as control.

Table 5. Results of Antimicrobial Test of JJJee against *Listeria monocytogenes*.

Test sample	Initial	Viable cell count (cfu /mL)	
		15 minutes	30 minutes
JJJee	$2.9 \times 10^6$	$1.6 \times 10^6$ (46.7%)	$1.6 \times 10^3$ (>99.9%)
Control	$2.9 \times 10^6$	$3.0 \times 10^6$	$3.1 \times 10^6$

Normal saline solution was used as control.

Table 6. Results of Antimicrobial Test of JJJee against *Candida albicans*.

Test sample	Initial	Viable cell count (cfu /mL)	
		2 hours	4hours
JJJee	$1.3 \times 10^6$	$1.2 \times 10^5$ (94.0%)	$3.9 \times 10^4$ (98.1%)
Control	$1.3 \times 10^6$	$2.0 \times 10^6$	$2.0 \times 10^6$

Normal saline solution was used as control.