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試 験 報 告 書

北生発 16_0121_2 号の英訳報告書

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試験内容を公表する際は、結果の表記等について専門的な立場から確認させていただいております。なお、確認目的と申込様式は、ホームページに収載しております。
(http://www.kitasato-e.or.jp/wordpress/?page_id=182)

To: Enagic Inc.

Study Report

Bactericidal Effects of Electrolyzed Water

Study Report No.16-0121_2
November 30, 2004

1-15-1 Kitasato, Sagamihara-shi, Kanagawa
Kitasato Research Center of Environmental Sciences
Cheif Director Haruo Tanaka

1. Study Objective
The present study was conducted to determine the bactericidal effects of electrolyzed water.

2. Client
Name: Enagic, Inc.
Address: 1-40-1 Hoshida Kita, Katano-shi, Osaka

3. Study Institution
Name: Kitasato Research Center of Environmental Sciences
Address: 1-15-1 Kitasato, Sagamihara-shi, Kanagawa
Study personnel: Ritsuko Kikuno and Akira Okaue, Biotechnology Section, microbiology Department

4. Materials
Apparatus: LeveLuk SD 501
Test water: Strongly acidic electrolyzed water produced using the above apparatus (samples collected after 10 minutes of passage through the apparatus)
Control water: Sodium hypochlorite solution

Test water conditions: See the table below.

Parameter	Strongly acidic electrolyzed water from LeveLuk SD 501	Sodium hypochlorite solution
pH	2.2	9.0
Redox potential (mV)	1164	726
Residual chlorine concentration (mg/L)	15.7	16.9

5. Test Organisms
1) *Staphylococcus aureus* NBRC 12732
2) *Staphylococcus aureus* ATCC 33591 (MRSA)
3) *Escherichia coli* ATCC 8739
4) *Escherichia coli* ATCC 35150 (O157)
5) *Pseudomonas aeruginosa* IFO 13275 (=NBRC 13275)
6) *Salmonella* serotype enteritidis IFO 3133 (=NBRC 3133)
7) *Legionella pneumophila* ATCC 33153
8) *Mycobacterium bovis* BCG RIMD 1314006 (bovine tuberculosis BCG strain)

6. Study Period
October 26 - November 26, 2004

7. Study Method
(1) Preparation of test water
The test apparatus was placed in a laboratory at Kitasato Research Center. With the water supply hose connected directly to a tap, city water was supplied and electrolyzed to obtain strongly acidic water, as specified in the operating procedures of the apparatus.

(2) Preparation of sodium hypochlorite solution
A sodium hypochlorite solution was diluted with distilled water to obtain the same concentration as the residual chlorine concentration of the test water.

(3) Bactericidal effects
Bacterial count of pre-cultured bacterial suspensions was adjusted to approximately 10^8 CFU/mL. To set up test samples, 10 mL each of the test water as well as the sodium hypochlorite solution was transferred into 50-ml test tubes and kept them at 20 ± 1℃. 0.1 mL each of the test microorganism suspensions was added to the tubes and incubated for 10 or 30 seconds with the exception of the case of bovine tuberculosis where the samples were incubated for 30 seconds, 10 minutes or 30 minutes.

After 0.1 ml of 3% sodium thiosulfate solution was added to the each sample to neutralize the chlorine ion activity at the end of the treatments, bacterial count of the samples was analyzed. Sterilized distilled water was used as negative control for initial bacterial count.

(4) Microorganisms counting
After residual chlorine neutralization, the test suspension (stock fluid) was prepared to obtain a series of 10-fold dilutions. A 0.1 mL portion of each of the stock fluid and the dilutions was applied to agar medium and cultured under the conditions for each organism.

The culture conditions for the individual organisms are shown in Table 1.

(5) Determination of residual chlorine concentrations
Residual chlorine concentrations were determined using the HACH Pocket Colorimeter 46700-00 model.

(6) Determination of pH and redox potential
pH was determined using the Horiba D-22 pH meter. Redox potential was determined using the Horiba D-54 pH meter.

8. Test Results
The bactericidal activity was shown in Tables 2~9.

Electrolyzed strong acidic water generated by LeveLuk SD501 showed bactericidal activity on the 7 species of test bacteria except for the bovine tuberculosis for 10 seconds incubation. This activity was equal to that of the same residual concentration of the sodium hypochlorite solution.

When incubated for 10 minutes, this electrolyzed strong acidic water was able to reduce the bovine tuberculosis count to less than 10 CFU/mL. On the other hand, the sodium hypochlorite solution required 30 minutes to obtain the same reduction rate.

Table 1: Test Organisms and Culture Conditions

Test organism	Preculture conditions	Cell counting conditions
Staphylococcus aureus *Staphylococcus aureus* (MRSA) *Escherichia coli* *Escherichia coli* O157 *Salmonella* serotype enteritidis *Pseudomonas aeruginosa*	Tryptic Soy Agar (Difco) At 35°C for 20 hours	Tryptic Soy Agar (Difco) At 35°C for 48 hours
Legionella pneumophila	BCYE α agar medium (Kyokuto) At 35°C for 3 days	BCYE α agar medium (Kyokuto) At 35°C for 5 days
Mycobacterium bovis	1% ogawa medium (Kyokuto) At 35°C for 4 weeks	7H11 agar medium (Difco) At 35°C for 4 weeks

Table 2: Determination of Bactericidal Effects of LeveLuk SD 501 (*Staphylococcus aureus)*

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Staphylococcus aureus NBRC 12732	Distilled water	1	2.1×10^6		
		2	2.0×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 3: Determination of Bactericidal Effects of LeveLuk SD 501 (Methicillin-resistant *Staphylococcus aureus*)

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Staphylococcus aureus (MRSA) ATCC 33591	Distilled water	1	2.4×10^6		
		2	2.4×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 4: Determination of Bactericidal Effects of LeveLuk SD 501 (*Escherichia coli*)

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Escherichia coli ATCC 8739	Distilled water	1	2.0×10^6		
		2	3.8×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 5: Determination of Bactericidal Effects of LeveLuk SD 501 (*Escherichia coli* O157)

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Escherichia coli 0157 ATCC 35150	Distilled water	1	1.7×10^6		
		2	1.6×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 6: Determination of Bactericidal Effects of LeveLuk SD 501 (*Pseudomonas aeruginosa*)

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Pseudomonas aeruginosa IFO 13275	Distilled water	1	1.5×10^6		
		2	1.2×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 7: Determination of Bactericidal Effects of LeveLuk SD 501 (*Salmonella* serotype enteritidis)

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Salmonella serotype enteritidis IFO 3133	Distilled water	1	2.2×10^6		
		2	2.3×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 8: Determination of Bactericidal Effects of LeveLuk SD 501 (*Legionella pneumophila*)

Test organism	Test water	CFU/mL			
		Sample number	0 seconds	10 seconds	30 seconds
Legionella pneumophila ATCC 33153	Distilled water	1	4.8×10^6		
		2	6.0×10^6		
	LeveLuk SD 501	1		<10	<10
		2		<10	<10
	Sodium hypochlorite solution	1		<10	<10
		2		<10	<10

Table 9: Determination of Bactericidal Effects of LeveLuk SD 501 (*Mycobacterium bovis* BCG)

Test organism	Test water	CFU/mL				
		Sample number	0 seconds	30 seconds	10 minutes	30 minutes
MJycobacterium bovis BCG RIMD 1314006	Distilled water	1 2	5.0×10^{5} 3.2×10^{5}			
	LeveLuk SD 501	1 2		8.8×10^{4} 8.4×10^{4}	<10 <10	<10 <10
	Sodium hypochlorite solution	1 2		2.5×10^{5} 3.0×10^{5}	9.5×10^{1} 1.4×10^{2}	<10 <10