

Disinfection effect of electrolyzed acid water against sporangium

Outline of experiment

Test report

Reported by
Dr. Atsuo Iwasawa
Tissue Culture Laboratory
Fujigaoka Hospital
Showa University
1-30 Fujigaoka, Aoba-ku, Yokohama City
Kanagawa, 227-8501, Japan

Outline of experiment

Two experiments have been performed to verify the disinfection effect of acid electrolyzed water against sporangium. In the first experiment, carrier inoculated with sporangium was inserted into a beaker containing acid electrolyzed water. As shown below, no disinfection effect had been recognized from this experiment. Acid electrolyzed water shows wide disinfection spectrum, disinfection effect in short period and strong disinfection, however, the disinfection effect would decrease when it was left for a long period and the effect would not continue. In addition, relatively high volume of acid water would be required for a given amount of fungus (Article by Dr. Atsuo Iwasawa, Clinical examination, Vol. 37, No.8, 1993). Therefore, it will be necessary to have appropriately controlled environment for applying acid electrolyzed water as disinfectant.

In the second experiment, mechanical properties had been taken into account, using endoscopes disinfector and CLEANTOP WM-S. In accordance with FDA AOAC Sporidical Test as far as possible, the carriers that had been inoculated with sporangium were inserted into an endoscopes disinfector, a disinfection tank filled with CLEANTOP WM-S, and the carriers were disinfected.

Experiment I (In vitro)

Kind of sporangium: Bacillus Subtilis JCM1465

Preparation of inoculation solution:

SCD culture medium ([Soybean-casein digest broth DAIGOD393-00185] made by Nihon Pharmaceutical Co., Ltd. and sold by Wako Junyaku Kogyo).

30 ml of the SCD culture medium was put into a tube of 50 ml (polypropylene) of NUNC make and inoculated with above fungus to culture for 3 days in an incubator (37±2°C). After having cultured, solution filtered with a stainless mesh (200 µm) was used for the test.

Preparation of carriers:

Sterilized stainless carriers, one piece per 1 ml fungus solution, were put into a beaker and left at room temperature for 15 minutes. After the given period, the carriers were transferred to a glass plate filled with sterilized filter paper and the excess of fungus solution was removed. The carriers were transferred to a new plate with sterilized new filter paper and left at room temperature until the next day.

Acid water generator: Oxilizer (made by Miura Electronics)

Specification of initial acid electrolyzed water: pH 2.7, more than ORP 1,100 mV, free chlorine 30~40 ppm

Test method (I):

50 ml of electrolyzed acid water was put into a 50 ml beaker with 5 pieces of the carriers soaked. After the periods specified below, each one carrier was put on SCDLP culture medium of 10 ml, and existence of fungus proliferated was observed after 2 days cultivation.

For information, the test was performed twice in parallel.

Time of soaking	Result (negative or positive)		Time of soaking	Result (negative of positive)	
(Group 1)	Sample 1	Sample 2	(Group 2)	Sample 1	Sample 2
5 min.	5/5	5/5	Mixing solution by stirrer		
10 min.	5/5	5/5	10 min.	5/5 (Fast stir)	5/5 (Slow stir)
20 min.	5/5	5/5	30 min.	5/5 (Fast stir)	5/5 (Slow stir)
40 min.	5/5	5/5	60 min.	5/5 (Fast stir)	5/5 (Slow stir)
60 min.	5/5	5/5			
5 min. x 2	5/5	5/5			
5 min. x 3	5/5	5/5			
10 min. x 2	5/5	5/5			
10 min. x 3	4/5	4/5			
Control	5/5	5/5			

Test method (II):

Instead of strong acid electrolyzed water, adjusted acid water of sodium hypochloride (Milton made by Kyorin Pharmaceutical Co., Lt.) and dilute hydrochloric acid was used. For comparison of disinfection effect against sporangium, pH depending on the concentration of sodium hypochloride 100 mg/l (100 ppm) was varied. The results are as follows:

Soaking time	10 min.		30 min.		60 min.	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
pH 2.5	5/5	5/5	5/5	5/5	5/5	5/5
pH 2.8	5/5	5/5	4/5	5/5	5/5	3/5
pH 2.9	5/5	5/5	3/5	4/5	2/5	3/5
pH 3.5	5/5	5/5	1/5	2/5	0/5	2/5
pH 5.5	5/5	5/5	1/5	2/5	0/5	1/5
pH 7.4	5/5	5/5	0/5	2/5	2/5	0/5
pH 9.2	5/5	5/5	5/5	5/5	0/5	4/5
Control	5/5	5/5	5/5	5/5	5/5	5/5

Experiment II

(Carriers were disinfected in CLEAN TOP WM-S)

Kind of sporangium: Bacillus Subtilis IFO 13722(ATCC #19659)

Preparation of inoculation solution:

Having inoculated the SCD culture medium with above fungus to culture for 3 days in an incubator ($37\pm 2^{\circ}\text{C}$). After having cultured, solution filtered with a stainless mesh (200 μm) was used for the test.

Preparation of carriers:

Ceramics carriers sterilized at 190 for 2 hours (dry high temperature process) were completely soaked in the fungus solution for 15 minutes at room temperature. After the given period, the carriers were taken out and transferred to a glass plate with sterilized filter paper. The excess of solution was removed from the plate and the carriers were put in a glass plate with sterilized new filter plate, and further into a desiccator with Silicagel. After suction for 20 minutes, it was left until the next day.

Culture medium:

Medium for judgments after processing was SCDLP medium [(Soybean-casein digest broth with lecithin & polysorbate 80 DAIGO*393-00185) made by Nihon Pharmaceutical Co., Ltd. and sold by Wako Junyaku Kogyo] and thioglycolic acid medium [(Thioglycollate medium*DAIGO*for JP general test 392-01211) made by Nihon Pharmaceutical Co., Ltd. and sold by Wako Junyaku Kogyo] was for sterilized test medium in electrolytic chamber.

Test method:

In accordance with the instructions to use on label of CLEAN TOP WM-S, 100 pieces of carriers were put into a disinfection tank. After each disinfection (3 minutes disinfection), 10 pieces of the carriers were taken out from the tank and were cultured.

The number of disinfections with CLEAN TOP WM-S was 20 and up to 10 times had been performed.

Culture period; 13 days and 21 days

- At the test, 1 ml of 100% mare's embryonic serum was inserted at the center of disinfection chamber. Total injection was 1 ml.
- At the test 2, no mare's serum was injected.
- At test 3, 0.5 ml of 100% mare's embryonic serum was injected at the center of the chamber just 'before every disinfection. Total injection was 5 ml.
- At the fifth disinfection of test 1, 0/9 shows that the carrier dropped on floor during collecting the 4 carrier and excluded the 1/10.
- To assure that the disinfection was complete, the electrolyzed acid water was taken out at completion of the fifth and tenth disinfections and cultured to see the existence of fungus. The amount of water taken out was 30 ml. Collecting fungus by means of centrifugal separator after the samplings, these were cultured in an incubator, after having removed the supernate and added with a sepsis testing culture liquid.

Time of soaking	Result (negative or positive)		Time of soaking	Result (negative or positive)	
(Group 1)	Sample 1	Sample 2	(Group 2)	Sample 1	Sample 2
5 min.	5/5	5/5	Mixing solution by stirrer		
10 min.	5/5	5/5	10 min.	5/5 (Fast stir)	5/5 (Slow stir)
20 min.	5/5	5/5	30 min.	5/5 (Fast stir)	5/5 (Slow stir)
40 min.	5/5	5/5	60 min.	5/5 (Fast stir)	5/5 (Slow stir)
60 min.	5/5	5/5			
5 min. x 2	5/5	5/5			
5 min. x 3	5/5	5/5			
10 min. x 2	5/5	5/5			
10 min. x 3	4/5	4/5			
Control	5/5	5/5			

Test method (II):

Instead of strong acid electrolyzed water, adjusted acid water of sodium hypochloride (Milton made by Kyorin Pharmaceutical Co., Lt.) and dilute hydrochloric acid was used. For comparison of disinfection effect against sporangium, pH depending on the concentration of sodium hypochloride 100 mg/l (100 ppm) was varied. The results are as follows:

Soaking time	10 min.		30 min.		60 min.	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
pH 2.5	5/5	5/5	5/5	5/5	5/5	5/5
pH 2.8	5/5	5/5	4/5	5/5	5/5	3/5
pH 2.9	5/5	5/5	3/5	4/5	2/5	3/5
pH 3.5	5/5	5/5	1/5	2/5	0/5	2/5
pH 5.5	5/5	5/5	1/5	2/5	0/5	1/5
pH 7.4	5/5	5/5	0/5	2/5	2/5	0/5
pH 9.2	5/5	5/5	5/5	5/5	0/5	4/5
Control	5/5	5/5	5/5	5/5	5/5	5/5

Test results

Test 1

Dis. cycle	Result		pH	ORP (mV)	CL Content (ppm)
	13 days	21 days			
1 cycle	10/10 *1	10/10	2.66	1120	10.9
2 cycle	5/10	5/10	2.58	1120	6.9
3 cycle	2/10	2/10	2.54	1125	7.5
4 cycle	0/10	0/10	2.50	1130	8.2
5 cycle	0/9 *4	0/9	2.44	1130	7.5
Chamber	Negative	Negative	*5 Collected sample from electrolysis chamber		
6 cycle	0/10	0/10	2.40	1130	6.8
7 cycle	1/10	1/10	2.34	1135	8.0
8 cycle	1/10	1/10	2.30	1135	7.6
9 cycle	2/10	2/10	2.26	1135	8.2
10 cycle	0/10	0/10	2.24	1140	8.3
Chamber	Negative	Negative	*5 Collected sample from electrolysis chamber		



Test 2

Dis. cycle	Result		pH	ORP (mV)	CL Content (ppm)
	13 days	21 days			
1 cycle	10/10	10/10	2.66	1120	10.6
2 cycle	5/10	6/10	2.60	1130	8.2
3 cycle	0/10	0/10	2.54	1130	8.2
4 cycle	0/10	0/10	2.50	1130	8.5
5 cycle	0/10	0/10	2.44	1135	8.7
Chamber	Negative	Negative	*5 Collected sample from electrolysis chamber		
6 cycle	0/10	0/10	2.40	1135	8.5
7 cycle	0/10	0/10	2.36	1140	8.3
8 cycle	0/10	0/10	2.30	1140	9.5
9 cycle	0/10	0/10	2.26	1140	9.1
10 cycle	0/10	0/10	2.44	1140	8.6
Chamber	Negative	Negative	*5 Collected sample from electrolysis chamber		

Test 3

Dis. cycle	Result		pH	ORP (mV)	CL Content (ppm)
	13 days	21 days			
1 cycle	9/10	9/10	2.60	1130	10.3
2 cycle	5/10	5/10	2.54	1130	7.8
3 cycle	2/10	2/10	2.50	1130	6.9
4 cycle	0/10	0/10	2.44	1125	5.9
5 cycle	0/10	0/10	2.40	1125	6.4
Chamber	Negative	Negative	*5 Collected sample from electrolysis chamber		
6 cycle	0/10	0/10	2.34	1125	4.6
7 cycle	1/10	1/10	2.30	1120	4.0
8 cycle	0/10	0/10	2.26	1120	3.8
9 cycle	1/10	1/10	2.22	1115	4.3
10 cycle	0/10	0/10	2.18	1115	4.1
Chamber	Negative	Negative	*5 Collected sample from electrolysis chamber		

Installation of carriers and sampling of them:

Carriers were evenly distributed in disinfection chamber. Some of them were flat and some were standing. Samples were selected equally from various locations.

Conclusion:

The disinfection effect with the soaking method in vitro was insufficient, however, it was confirmed that disinfection effect in the disinfectant method utilizing the properties of acid water (with continuously supplied acid water and increased chances to contact fungus with flowing water) showed sufficient effect. Therefore, the conventional evaluation method in vitro for disinfectants seems not to be suitable for electrolyzed acid water.