

Mycobacterium disinfection test with Cleantop WM®-S

Report

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Introduction:

We are preparing to report the disinfective activity of electrolyzed strong acid water containing 5 ppm free-chlorine against Mycobacteria. In the report we demonstrated that contaminated protein such as horse serum inhibit the activity against the bacteria, and suggested that stream of fresh electrolyzed strong acid water may bactericidal activity against Mycobacteria. In this report, to clarify whether the stream of fresh electrolyzed strong acid water reveals bactericidal activity, we constructed a small-scale experimental device and investigated the bactericidal activity against Mycobacterium bovis BCG.

Materials and Methods:

Experimental device: Sketch of an experimental device is shown in Fig. 1. The device is constructed with three parts: electrolyzing well, circulating pump and dipping chamber in which a testing acrylic tube is equipped. Flow of electrolyzed acid water is presented with arrows. The stream of electrolyzed strong acid water starts from the electrolyzing well, in which positive electrode is installed, to the experimental chamber through two directions. The stream contacts with the inside and outside of acrylic tube in the chamber, exists through the bottom of the experimental chamber, return to the electrolyzing well and electrolyzed again in the well. The stream of the water is closed in the circulation.

Preparation of an artificially contaminated acrylic tube: Mycobacterium avium grown on Ogawa slants was harvested, suspended in physiological saline containing 10% horse serum, vortexed and allowed to stand for 30 min at room temperature. The supernatant of the suspension was adjusted at McFarland 2. A testing tube was dipped in the supernatant for 20 min, and fixed in experimental chamber of the device. The device was run for 3 and 7 minutes at 10 ppm and 5 ppm respectively, and samples were harvested from outside and inside of testing tube were by wiping with a sterilized cotton swabs, respectively. The samples were inoculated to Ogawa slant and incubated for 4 weeks at 37°C. When visible colonies were detected on a slant, a sample was determined to be positive for residual bacteria. The experiments were duplicated. For the examination of water from the tank, 50 ml of the water was harvested from the tank and concentrated by centrifugation, and inoculated to Ogawa slant and examined as described above.

Results and Discussion:

Representative photograph of examined culture was shown in Fig. 2, and summarized data was presented in Table 1. Mycobacterium was detected outside and inside of the testing acrylic tubes that untreated and treated with unelectrolyzed NaCl solution. No Mycobacterium was detected in the tank of the device. We could not detect the bacterium in tank of unelectrolyzed situation. The unsuccessful detection may be caused by dilution effect, that is the concentration of water from the tank was not sufficient. Further experiments are needed on the survival of the bacterium in the tank. By the treatment with electrolyzed strong acid water containing 5 ppm and 10 ppm free chlorine, the bacterium could not be detected. These results indicated that the device may be effective to eliminate Mycobacteria contaminating outside and inside of smooth-surfaced tube.

Fig. 1: Sketch of experimental device

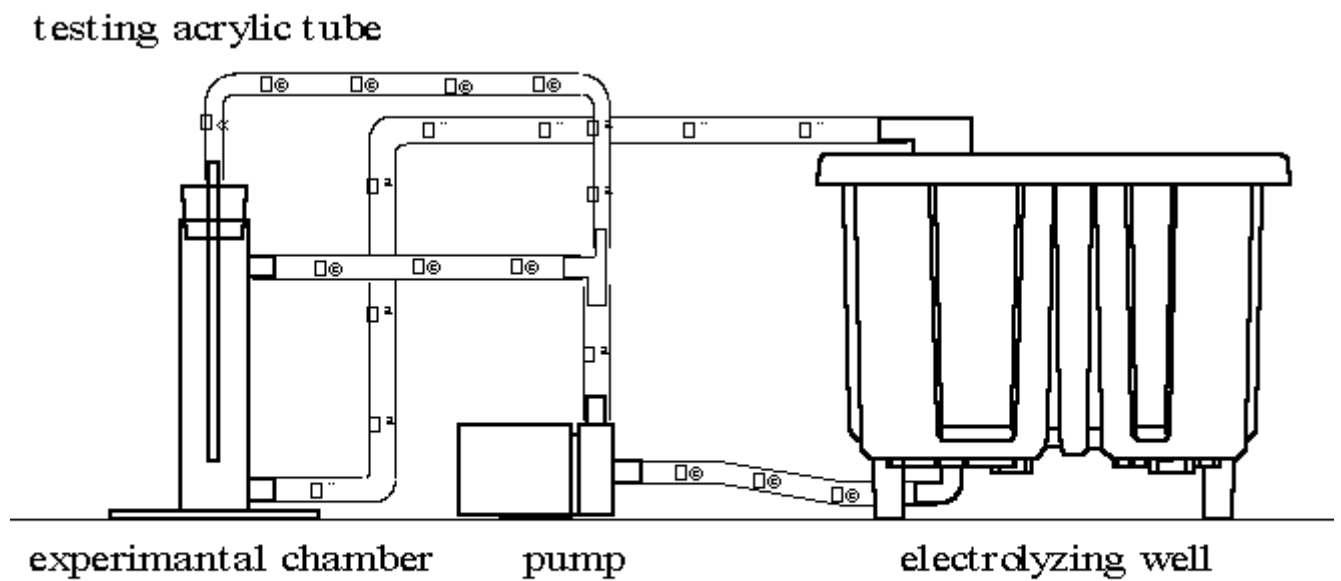


Fig.2: Representative photograph of growth of Mycobacterium avium, BCG treated with electrolyzed strong acid water.

untreated		0.1% NaCl			5 ppm			10 ppm		
out	in	out	in	tank	out	in	tank	out	in	tank



untreated: artificially contaminated acrylic tube; out: outside of an acrylic tube; in: inside of an acrylic tube; 0.1%NaCl: unelectrolyzed 0.1% NaCl solution; 5 ppm: electrolyzed strong acid water containing 5 ppm free chlorine; 10 ppm: electrolyzed strong acid water containing 10 ppm free chlorine

Table 1: Growth of Mycobacterium bovis, BCG

Treated with	Outside	Inside	Tank
0.1 % NaCl	+	+	-
5 ppm	-	-	-
10 ppm	-	-	-
untreated	+	+	UT

Remarks :

The device was run for 3 , 7 and 7 minutes at 10 ppm CL content , 5 ppm CL content and 0.1% NaCL solution respectively.

untreated: artificially contaminated acrylic tube; outside: outside an acrylic tube; in: inside an acrylic tube; tank: water from electrolyzing tank; 0.1%NaCl: unelectrolyzed 0.1% NaCl solution; 5 ppm: electrolyzed strong acid water containing 5 ppm free chlorine; 10 ppm: electrolyzed strong acid water containing 10 ppm free chlorine; UT: untestable