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Chlorine Inactivation of Bacterial Bioterrorism Agents

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Seven species of bacterial select agents were tested for susceptibility to free available chlorine (FAC). Under test conditions, the FAC routinely maintained in potable water would be sufficient to reduce six species by 2 orders of magnitude within 10 min. Water contaminated with spores of *Bacillus anthracis* spores would require further treatment.

The contamination of the U.S. mail system with anthrax spores in 2001 has heightened concern about the safety of other public services and utilities, including our water distribution systems. Currently, chlorination is the most common method of disinfecting drinking water in the United States (2, 3). The U.S. Environmental Protection Agency and the water treatment industry use Ct values ("C" is the chlorine concentration in milligrams/liter, and "t" is the exposure time in minutes) to calculate microbial inactivation and to evaluate the effectiveness of a water treatment system. Ct tables have been developed for some waterborne pathogens to indicate conditions necessary for a 2-$\log_{10}$ (99%) or 3-$\log_{10}$ (99.9%) inactivation. The present study determined Ct values for 2-log and 3-log inactivation of seven species (11 isolates) on the Centers for Disease Control and Prevention (CDC) list of category A and B potential bioterrorism agents (12).

The bacterial strains *Bacillus anthracis* Ames, *Brucella melitensis* ATCC 23456, *Brucella suis* EAM562, *Burkholderia mallei* M-9, *Burkholderia mallei* M-13, *Burkholderia pseudomallei* ATCC 1688, *Francisella tularensis* LVS, *Francisella tularensis* NY98, *Yersinia pestis* A1122, and *Yersinia pestis* Harbin were obtained from CDC laboratories. *B. anthracis* Sterne 34F2 was obtained from Colorado Serum Co., Denver, Colo.

The effect of each chlorine concentration was tested in triplicate by using chlorine demand-free buffer (0.05 M KH_2PO_4; pH 7) and maintained at 5 and 25°C. Testing methods are described elsewhere (4). Free available chlorine (FAC) and total chlorine were monitored by using DPD colorimetric analysis (1).

Decay curves were generated for each organism by using the $\log_{10}$-transformed data of the mean CFU counts at each time, temperature, and chlorine concentration. Linear regressions of the appropriate segments of the decay curves were performed to estimate the time needed for a 99 or 99.9% reduction in viable counts. The Ct values were calculated by multiplying inactivation times for a given temperature and percent inactivation by the chlorine concentration at that time. The reported Ct values represent the mean of the Ct values calculated for each chlorine concentration.

The results of the chlorine challenge and the calculated Ct values are shown in Table 1 for the gram-negative bacteria and in Table 2 for *Bacillus anthracis* spores. *Burkholderia*, *Brucella*, and *Yersinia* strains were more susceptible to chlorine treatment than *Francisella tularensis*, as shown by Ct values ≤0.7 for a 3-log inactivation of these organisms. Ct values for a 3-log inactivation of *Francisella tularensis* ranged from 1.0 to 10.3. The slightly greater resistance of *Francisella tularensis* to chlorine was also observed by Foote et al. (6) as determined by injection into guinea pigs.

The *Bacillus anthracis* spores were less susceptible to chlorine disinfection than the gram-negative organisms. The Ames strain was slightly less susceptible to the chlorine than the Sterne strain, requiring more than 2 h for a 2-log reduction when exposed to 0.8 mg of FAC/liter at 25°C, whereas the Sterne strain underwent a >4-log reduction in counts after 2 h under similar conditions (Table 2). The Ct values determined in the present study for *B. anthracis* spores are comparable to the data obtained by Brazis et al. (5), from which Ct values can be calculated to be 458 at 4°C and 113 at 22°C for a 4-log reduction (99.99%). Differences between the Brazis findings and the results of the present study may be attributed to variability between strains, slight pH or temperature differences, or methods of spore preparation.

A 1992 survey of samples from 283 water utilities reported that of those that use chlorine, a median residual of ~1.1 mg/liter, and a median contact time to the first point of use (from treatment facility to first access point in the water distribution system) of 45 min was reported in the utilities responding to the survey (13). Using the survey median result as a guide, we can estimate a median Ct value of 49.5 (1.1 mg/liter × 45 min) for the 283 water utilities surveyed. Our study shows that viable *Burkholderia mallei*, *Burkholderia pseudomallei*, *Brucella melitensis*, *Brucella suis*, *Francisella tularensis*, and *Yersinia pestis* would be reduced by more than 3 orders of magnitude under these median conditions if pH and temperatures were similar to those in the present study. The *Bacillus anthracis* spores, however, would not be inactivated by 2 $\log_{10}$ or 3 $\log_{10}$ under these median treatment conditions.

The Ct value can provide some indication of the efficacy of

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TABLE 1. Free chlorine inactivation of select agents at pH 7

Isolate	Temp (°C)	Initial inoculum (log_{10} CFU)	FAC (mg/liter)	Viable cells after exposure time [mean log_{10} (CFU + 1)/ml, $n = 3$][a] of:			Ct (mg min/liter)	
				1 min	5 min	10 min	2 log	3 log
Burkholderia mallei M-9	5	5.8	0.14	5.1	0.5	0.0	0.2	0.2
			0.39	0.0	0.0	0.0		
	25	6.0	0.15	0.8	0.0	0.0	0.1	0.2
			0.46	0.0	0.0	0.0		
Burkholderia mallei M-13	5	5.8	0.13	5.7	2.1	2.1	0.2	0.2
			0.47	0.0	0.0	0.0		
	25	5.7	0.21	0.0	0.0	0.0	0.1	0.2
			0.60	0.0	0.0	0.0		
			1.01	0.0	0.0	0.0		
Burkholderia pseudomallei ATCC 1688	5	5.5	0.21	3.2	1.6	0.0	0.5	0.7
			0.65	2.3	0.0	0.0		
			1.43	0.0	0.0	0.0		
	25	5.6	0.08	4.4	2.6	0.9	0.4	0.6
			0.43	1.5	0.0	0.0		
			0.85	0.0	0.0	0.0		
Brucella melitensis ATCC 23456	5	6.6	0.16	3.6	2.9	2.5	0.3	0.5
			0.50	3.0	2.0	0.5		
			1.10	2.4	0.4	0.0		
	25	6.9	0.04	4.8	2.6	1.8	0.1	0.2
			0.23	0.8	0.4	0.0		
			0.85	0.0	0.0	0.0		
Brucella suis EAM562	5	6.8	0.20	2.6	1.2	0.0	0.3	0.4
			0.50	2.3	0.0	0.0		
			1.00	0.0	0.0	0.0		
	25	7.2	0.15	1.1	0.0	0.0	0.1	0.2
			0.20	0.1	0.0	0.0		
			0.60	0.0	0.0	0.0		
Francisella tularensis NY98	5	6.5	0.20	6.4	6.2	6.1	7.8	10.3[b]
			0.54	6.4	6.1	5.8		
			1.00	6.3	5.9	4.2		
	25	6.6	0.30	6.1	6.2	5.6	2.0	3.9
			0.72	5.5	4.1	0.0		
			1.66	3.7	1.9	0.0		
Francisella tularensis LVS	5	7.0	0.23	5.5	4.9	3.9	1.5	2.4
			0.50	5.2	3.6	1.9		
			1.06	4.8	2.7	1.3		
	25	6.8	0.10	5.5	5.5	5.5	0.6	1.0
			0.36	5.4	1.9	2.1		
			0.86	3.7	0.0	0.0		
Yersinia pestis A1122	5	6.1	0.42	0.0	0.0	0.0	0.5	0.7
			0.98	0.0	0.0	0.0		
			1.84	0.0	0.0	0.0		
	25	6.4	0.37	1.0	0.0	0.0	0.4	0.6
			0.76	1.2	0.0	0.0		
			1.60	0.0	0.0	0.0		
Yersinia pestis Harbin	5	6.6	0.06	1.8	0.7	0.1	0.03	0.04
			0.31	0.0	0.0	0.0		
			0.84	0.0	0.0	0.0		
	25	6.6	0.08	1.6	0.0	0.0	0.03	0.04
			0.31	0.0	0.0	0.0		
			0.82	0.0	0.0	0.0		

[a] To account for zero values, we added 1 to all datum points before conversion to the log_{10} scale.
[b] Extrapolated value.

disinfectants on pathogenic organisms but must be determined for each species and at several temperatures and pH conditions. If the water temperature were lower or the pH were higher within a particular distribution system, the Ct values would be expected to be higher for all organisms than those calculated in the present study. Other factors that may promote survival of bacteria in chlorinated water include nutrient availability before exposure to FAC (9–11), attachment to surfaces (8), clumping and incorporation in organic materials (7), and incorporation into biofilms present in drinking water sys-

TABLE 2. Free chlorine inactivation of *Bacillus anthracis* spores at pH 7

Isolate	Temp (°C)	Initial inoculum (log_{10} CFU/ml)	FAC (mg/liter)	Viable spores after exposure time [mean log_{10} (CFU + 1)/ml, $n = 3$][a]				Ct (mg min/liter)	
				1 h	2 h	3 h	4 h	2 log	3 log
Bacillus anthracis Sterne	5	4.1	0.88	3.8	3.8	3.6	2.5	190	271
			1.98	3.6	2.6	0.2	0.0		
			2.94	2.7	0.0	0.0	0.0		
	25	4.3	1.02	3.6	0.0	0.0	0.0	60	86
			1.96	0.0	0.0	0.0	0.0		
Bacillus anthracis Ames	5	4.9	0.80	4.9	4.8	ND	3.9	220	339
			2.80	4.2	0.0	ND	0.0		
	25	4.9	0.80	4.6	2.6	ND	0.0	79	102
			2.80	0.0	0.0	ND	0.0		
			4.80	0.0	0.0	ND	0.0		

[a] To account for zero values, we added 1 to all datum points before conversion to the log_{10} scale.

tems (9). Continued work to address the efficacy of FAC under various water conditions, as well as evaluation of other water treatment methods such as monochloramine, ozone, and UV light, is essential for protecting public health in the event of an intentional release of these bacterial agents into a potable water system.

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