

technical **BRIEF**

Inactivation of Bacterial Bioterrorism Agents in Water: Summary of Seven Studies

INTRODUCTION

In the United States, chlorine and monochloramine are the primary chemical disinfectants used to inactivate microbes in drinking water distribution systems. Although many microbes are inactivated by common water treatments, some are more resistant. Conditions for inactivating many waterborne disease-causing microbes have been established, but there are only limited data on inactivating bacterial bioterrorism agents. U.S. EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.

U.S. EPA and the Centers for Disease Control and Prevention (CDC) have conducted seven laboratory-

based inactivation studies in water using non-disease causing surrogates for *Bacillus anthracis* and microbes identified as potential bioterrorism agents. One of the studies also examined the conditions under which boiling water could inactivate microscopic resistant structures (spores) formed by surrogates.

A number of factors influence the effectiveness of chemical disinfectants in drinking water treatment systems, including:

- the type and quantity of microbes present
- whether the microbes form spores or exist primarily as vegetative cells
- the type of disinfectant and its concentration
- the amount of time the disinfectant is in contact with the microbes
- water temperature
- water acidity or alkalinity (pH)
- the type and quantity of organic and inorganic particles in the water
- water flow and pipe materials

Different species and strains of bacteria, whether bioterrorism agents or not, can have different degrees of resistance to disinfectants. If nutrients are available prior to inactivation treatments, this can increase the resistance of some species to chemical disinfection. Clumping or attachment to floating organic materials can increase resistance. Some strains produce material outside their cell wall. This extracellular material can permit attachment to other organisms or surfaces and help form biologically active layers (biofilms), which are generally more resistant to chemical disinfection than free floating (planktonic) cells.

Conditions in the inactivation studies were controlled. The data obtained, while suggestive, cannot be directly applied to water distribution systems without factoring in circumstances that will affect the how long a specific disinfectant and its residuals will be in contact with the microorganisms.

OVERVIEW OF STUDIES ON THE INACTIVATION OF BIOTERRORISM AGENTS AND SURROGATES IN WATER

The agents investigated can cause diseases in humans or animals from one or more of these exposure routes [see References, Inactivation Studies 1, 2, 3, 5, 6, 7]:

- Direct contact with mucous membranes or broken skin
- Ingestion of contaminated food or water
- Inhalation of contaminated aerosols, dust, or particles

All of the agents have been investigated as possible biological weapons in the state-sponsored research of one or more countries. Some have been used as biological weapons. All are considered inhalation threats. In addition, four spore-forming surrogates, which are used by many researchers in place of the virulent *Bacillus anthracis* Ames, were investigated: *B. anthracis* Sterne; *B. cereus; B. globigii; B. thuringiensis* subsp. *israelensis* [see References, Inactivation Studies 3, 4, 5, 6, 7].

The two common inactivation methods in water (chlorine and monochloramine) were tested on 26 strains of the following seven bioterrorism agents:

Bacterial Agent	Disease Caused	CDC Category	On Select Agents List
Bacillus anthracis ^a	anthrax	А	HHS/APHIS
Brucella melitensis	brucellosis	В	HHS/APHIS
Brucella suis	brucellosis	В	HHS/APHIS
Burkholderia mallei	glanders	В	HHS/APHIS
Burkholderia pseudomallei	melioidosis	В	HHS/APHIS
Francisella tularensis	tularemia	А	HHS
Yersinia pestis	plague	А	HHS

^a forms spores

Bacterial agents are classified, transported, handled, and tested according to definitions and regulations

<u>CDC Category</u> - The Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services, categorize bioterrorism agents and diseases based on the degree to which they pose a national security risk. Highest-priority agents (Category A) can be easily spread in the environment or from person-to-person, result in high death rates, would potentially cause panic and social disruption, and require special public health preparedness. Category B agents are moderately easy to spread in the environment, result in a moderate number of illnesses and low death rates, and require some changes to public health preparedness.

<u>On Select Agents List</u> – The Select Agents and Toxins List is defined by the Centers for Disease Control and Prevention, U.S. Department of Health and Human Services (HHS) and the Animal and Plant Health Inspection Service, U.S. Department of Agriculture (APHIS) and lists biological agents or toxins deemed a threat to the public, animal or plant health, or to animal or plant products. There are regulations on handling, transporting, and using select agents for research and testing. Table 1 summarizes the bacterial bioterrorism agent strains and treatments with the reference numbers of the studies.

Table 1 Inactivation Studies of Bioterrorism Agents

				on Method Number of Study
Agents	Strains Tested		Chlorine	Monochloramine
Bacillus anthracis ^a	Ames		6 ^b	5
Brucella melitensis	ATCC 23456 ^C [f	NCTC 10094] ^d	6	5
Brucella suis	EAM562 MO562		6	
Burkholderia mallei	M-9 M-13	M-9		2
Burkholderia pseudomallei	ATCC 11668 [NCTC 11642] ATCC 23343 [NCTC 12939] ATCC 1688 [NCTC 1688] ATCC 1688] ATCC 1688] ATCC 1688] ATCC 1688] ATCC 1688] AU 631 (soil) CA 650 [ART] ^e CA 652 [ART] KC 872 SC 763 [ART] SC 764 [764] TH 694 (water)		2, 6	2, 5
Francisella tularensis	subsp. <i>holarctica</i> KY99-3387 (type B) subsp. <i>holarctica</i> LVS (type B) (vaccine) subsp. <i>holarctica</i> NY98 subsp. <i>holarctica</i> OR96-0246 (type B) subsp. <i>tularensis</i> Schu S4 (A1) subsp. <i>tularensis</i> WY96-3418 (A2) subsp. <i>tularensis</i> MA00-2987 (A1) subsp. <i>tularensis</i> NM99-1823 (A2)		1, 6	5
Yersinia pestis	A1122 (low virule Harbin	ence)	6	5

^a *B. anthracis* Ames and its surrogates form spores, the other bacteria in the inactivation studies live in vegetative cell stages, which are less resistant to inactivation; *B. anthracis* surrogates are itemized in Table 2.

^b Study [6] has the original experimental data derived at 25 °C, which is cited in studies [3] and [7] with the temperature listed as 23 °C.

^c ATCC and associated number are registered or nonregistered trademarks of the American Type Culture Collection, Manassas, Virginia, USA.

^d [NCTC] — the strain currently is listed by the National Collection of Type Cultures, Health Protection Agency, Salisbury, UK, but no longer listed in the ATCC.

e [ART] – is from the "Antimicrobials Resistance Team, CDC" (see reference [2])

Table 2 summarizes the surrogates for *B. anthracis* and treatments with the reference numbers of the studies.

Table 2 Inactivation Studies of Surrogates for Bacillus anthracis Ames

	Inactivation Method and Reference Number					
Bacteria Used as Surrogates for Bacillus anthracis Ames	Boiling in Tap Water	Free Available Chlorine	Monochloramine			
Bacillus anthracis Sterne 34F2 [NCTC 8234] a	4	3, 6	5			
Bacillus cereus	4 (ATCC 9592 ^b)	3 (ATCC 7039 ^b)				
Bacillus cereus (commercial)	4					
<i>Bacillus globigii</i> (Dugway) ^c		7				
Bacillus thuringiensis subsp. israelensis ATCC 35646	4	3				

 ^a [NCTC] — the strain currently is listed by the National Collection of Type Cultures, Health Protection Agency, Salisbury, UK.
 ^b ATCC and associated number are registered or nonregistered trademarks of the American Type Culture Collection, Manassas, Virginia, USA.

^c Strain from the U.S. Army Dugway Proving Ground, Utah

RESULTS FROM THE CHEMICAL INACTIVATION STUDIES [1, 2, 3, 5, 6, 7]

A chemical disinfectant must be in contact with organisms for the length of time needed to inactivate them and keep them inactivated. The condition needed for inactivation is expressed as the Ct value (mg*min/L).

The Ct value is derived from experimental data and represents the disinfectant concentration (C, in milligrams per liter) multiplied by the contact time (t, in minutes). Ct values are used to establish the required conditions needed to achieve the desired amount of inactivation (log_{10} reduction) for a particular microorganism under a specific temperature and pH. Different concentration and contact time combinations can result in the same Ct value.

Conditions that have an effect on the values include:

- the number and characteristics of the microbes,
- water temperature and pH
- quantity of suspended or attached particles
- disinfectant concentration
- water treatment system infrastructure (pipe materials, pipe loop designs, and age)

The typical conditions for chlorine (free available chlorine or FAC) and monochloramine - At the time the inactivation studies were conducted, the conditions for chlorine treatment ^a at surveyed water treatment plants were a median FAC of 1.1 mg/L and a median contact time from the treatment facility to the drinking water customer of 45 minutes. For monochloramine treatment ^b, the conditions were a median target concentration of 2 mg/L and a median contact time from facility to customer of 45 minutes.

^a Water Quality Disinfection Committee. 1992. <u>Survey of water utility disinfection practices</u>. J. Am. Water Works Assoc. 84(9): 1-128.
 ^b Seidel, C.J., McGuire, M.J., Summers. R.S., and Via, S. 2005. <u>Have utilities switched to chloramines?</u> J. Am. Water Works Assoc. 97(10): 87-97.

Table 3 summarizes either the highest Ct value (mg*min/L) for a species that had multiple strains tested in one or more studies (= highest) or the only Ct value (mg*min/L) observed for a species and strain in only one study (= single). These Ct values are indicative of the efficacy of the disinfectants on a particular species and strain under particular temperatures and pH values.

Table 3 Summary of Bioterrorism Agent and Surrogate Inactivation Results Expressed asCt Values

Microorganisms Tested	Strains With Highest or Single	Temperature •C	Highest or Single Ct (mg*min/L) Values for 3 log ₁₀ Inactivation		
	Ct (mg*min/L) Values	Temp	Chlorine pH 7	Mono- chloramine pH 8	
		5	339	6,813	
	Ames	15		1,691	
Bacillus anthracis		25	102	1,204	
		5	271	15,164	
	Sterne 34F2	15		3,925	
		25	86	1,847	
Bacillus thuringiensis subsp. israelensis ATCC 35646		5	175		
		23	62		
		5	446		
Bacillus thuringiensis subsp. israelensis ATCC 356	(Dugway)	23	136		
Bacillus thuringiansis subsp. israelonsis		5	344		
Bacilius thuringiensis subsp. israelensis	ATCC 30040	23	99		
		5	0.5	579.5	
Brucella melitensis	ATCC 23456	15		223.9	
		25	0.2	116.6	
	EAM562	5	0.4		
		25	0.2		
Brucella suis		5		156.8	
	MO562	15		120.4	
		25		56.1	
		5	0.2	194.1	
	M-9	15		102.4	
Burkholderia mallei		25	0.2	64.6	
	M-13	5	0.2		
		25	0.2		
	CA 652 (mucoid clinical)	5	3.7		
Burkholderia pseudomallei		25	1.3		
	TH 694 (water)	5		477	
		25		113	
	KY99-3387 (type B)	5	18.3		
	())/	25	1.0		
Francisella tularensis subsp. holarctica		5	10.3 ^a	116.0	
	NY98 (type B)	15		64.8	
		25	3.9	37.1	
		5	0.7	115.6	
Yersinia pestis	A1122	15		86.4	
		25	0.6	33.1	

---- not tested under these conditions ^a extrapolated, see reference [6]

Summary of the Major Conclusions from Studies on Chemical Inactivation of Bioterrorism Agents [1,2,5,6]

Agent	Under Typical Free Available Chlorine Conditions ^a	Under Typical Monochloramine Conditions ^b
	[6] <i>B. anthracis</i> would not be inactivated by a 2 log ₁₀ reduction	[5] depending on the temperature, Ct values for <i>B.</i> <i>anthracis</i> Sterne [surrogate] were 1.5 to 3 times greater than those of Ames [virulent strain]
Bacillus anthracis	[6] "The Ames strain was slightly less susceptible to the chlorine than the Sterne strain, requiring more than 2 h for a 2 log ₁₀ reduction when exposed to 0.8 mg/L FAC at 25 °C, whereas the Sterne strain underwent a > 4 log ₁₀ reduction in counts after 2 h under similar conditions."	[5] <i>B. anthracis</i> Ames spores cannot be reduced by 2 or 3 log ₁₀ "regardless of temperature and would require hours or days of disinfectant exposure"
Brucella spp.	 [6] <i>B. suis</i> EAM562 and <i>B. melitensis</i> ATCC 23456 would be reduced by 3 log₁₀ if "pH and temperature were similar to those in the present study" {5 and 25 °C (41 and 77 °F) and pH 7} 	[5] <i>B. suis</i> MO 562 and <i>B. melitensis</i> ATCC 23456 "would require a longer contact time or higher disinfectant concentration for a 2 log ₁₀ reduction"
Burkholderia mallei	[6] <i>B. mallei</i> M-9 and M-13 would be reduced by 3 log ₁₀ if pH and temperature were similar to those in the present study" {5 and 25 °C (41 and 77 °F) and pH 7}	[5] <i>B. mallei</i> M-9"demonstrated a 2 log ₁₀ inactivation" at a Ct value of 52.5 at 25 °C
	{Strains tested in [2] <i>B. pseudomallei</i> AU 631; TH 694; SC 763; SC 764; ATCC 11668; ATCC 23343; CA 650; CA 652}	{Strains tested in [2] for monochloramine were <i>B. pseudomallei</i> AU 631; TH 694; SC 763; SC 764; ATCC 11668; ATCC 23343; CA 650; CA 652}
	 [2] the planktonic populations of tested strains of <i>B. pseudomallei</i> could be reduced by 4 log₁₀ (in less than 10 minutes) 	[2] the planktonic populations of tested strains of <i>B. pseudomallei</i> could be not reduced by 4 log ₁₀
	[2] For a 3 log ₁₀ reduction, an 18-fold difference was seen between the Ct values of the most and least resistant strains (Ct values ranged from 0.2 to 3.7 mg*min/L at pH 7 and	[2] "Ct values were less variable than FAC Ct values, differing by a factor of 2.5 between tested strains"
Burkholderia	5 °C (41 °F)	[2] Ct values were independent of the amount of extracellular material produced by each strain
pseudomallei	[2] The relative sensitivity to chlorine was determined to be independent of a strain's environmental, clinical, or culture collection origins	[5] <i>B. pseudomallei</i> KC 872 would be reduced by 2 log ₁₀
	[2] The relative amount of extracellular material produced by a strain increased its tolerance to chlorine	
	[6] <i>B. pseudomallei</i> ATCC 1688 would be reduced by 3 log ₁₀ if "pH and temperature were similar to those in the present study" {5 and 25 °C (41 and 77 °F) and pH 7}	

^a Median FAC of 1.1 mg/L and a median contact time from the treatment facility to the drinking water customer of 45 minutes ^b Median target concentration was 2 mg/L and a median contact time from the treatment facility to the drinking water customer of

Summary of the Major Conclusions from Studies on Chemical Inactivation of Bioterrorism Agents [1,2,5,6]continued

Agent	Under Typical Free Available Chlorine Conditions ^a	Under Typical Monochloramine Conditions ^b
	{Strains tested in [1] for FAC were, <i>F. tularensis</i> subsp. <i>holarctica</i> KY99-3387 (type B) , LVS (type B), and OR96-0246 (type B) and <i>F. tularensis</i> subsp. <i>tularensis</i> MAOO-2987 (A1), NM99-1823 (A2), Schu S4 (A1), and WY96-3418 (A2)}	[5] <i>F. tularensis</i> subsp. <i>holarctica</i> LVS (type B) and <i>F. tularensis</i> subsp. <i>holarctica</i> NY 98 (type B) could be reduced by 3 log ₁₀ if the temperature of the water was 15 °C (59 °F) or higher and the pH maintained at 8
	[1] A 4 log ₁₀ reduction of viable <i>F. tularensis</i> counts occurred most rapidly at 25 °C (77 °F) and pH 7; there was no significant difference between the Ct values for all tested strains under these conditions; disinfection occurred most slowly at pH 8 and 5 °C (41 °F)	
	[1] For all conditions other than pH 7 and 25 °C (77 °F), the live vaccine strain, <i>F. tularensis</i> subsp. <i>holarctica</i> LVS (type B), was more sensitive to chlorine than the other strains.	
Francisella tularensis	[1] The study recommended, when possible, using Type B strains with full virulence, rather than the live vaccine strain to avoid underestimating Ct values needed for disinfection	
	 [1] The most favorable temperature 25 °C (77 °F) and pH 7 combination would reduce the planktonic population of the most sensitive strain by 4 log₁₀ in less than one minute 	
	[1] The least favorable temperature (5 °C (41 °F) and pH 8 would require up to 1.7 hours to reduce the planktonic population of the most tolerant strain by 4 log ₁₀	
	[[6] <i>F. tularensis</i> subsp. <i>holarctica</i> LVS (type B) and <i>F. tularensis</i> subsp. <i>holarctica</i> NY 98 (type B) would be reduced by 3 log ₁₀ if "pH and temperature were similar to those in the present study" {5 and 25 °C (41 and 77 °F) and pH 7}	
Yersinia pestis	[6] <i>Y. pestis</i> A1122 and Harbin would be reduced by 3 \log_{10} if "pH and temperature were similar to those in the present study" {5 and 25 °C (41 and 77 °F) and pH 7}	[5] <i>Y. pestis</i> A1122 and Harbin could be reduced by 3 log ₁₀ under median conditions if the temperature of the water was 15 °C (59 °F) or higher and the pH maintained at 8

^a Median FAC of 1.1 mg/L and a median contact time from the treatment facility to the drinking water customer of 45 minutes

^b Median target concentration was 2 mg/L and a median contact time from the treatment facility to the drinking water customer of 45 minutes

Summary of the Major Conclusions from Studies on Chemical Inactivation of Surrogates [3,5,6,7]

Surrogates for Bacillus anthracis	Under Typical Free Available Chlorine Conditions ^a	Under Typical Monochloramine Conditions ^b
<i>Bacillus anthracis</i> Sterne 34F2	[3] Spores of <i>B. anthracis</i> Sterne had "substantially lower Ct values" than the spores of <i>B. thuringiensis</i> subsp. <i>israelensis</i> or of <i>B.</i> <i>anthracis</i> Ames	[5] <i>B. anthracis</i> Sterne spores cannot be reduced by 2 or 3 log ₁₀ "regardless of temperature and would require hours or days of disinfectant exposure"
<i>Bacillus cereus</i> ATCC 7039	[3] Spores of <i>B. cereus</i> had "substantially lower Ct values" than the spores of <i>B. thuringiensis</i> subsp. <i>israelensis</i> or of <i>B. anthracis</i> Ames	
Bacillus thuringiensis subsp. israelensis ATCC 35646	[3] "Spores of <i>B. thuringiensis</i> subsp. <i>israelensis</i> would be an appropriate surrogate to use in place of <i>B. anthracis</i> in chlorine inactivation studies"	

^a Median FAC of 1.1 mg/L and a median contact time from the treatment facility to the drinking water customer of 45 minutes ^b Median target concentration was 2 mg/L and a median contact time from the treatment facility to the drinking water customer of 45 minutes

RESULTS FROM THE BOILING INACTIVATION STUDY [4]

Results of this study have implications for boil water advisories that are issued by public health and other authorities. Many waterborne, disease-causing microbes can be inactivated at a rolling boil held for one minute. However, even after five minutes of boiling in tap water in an uncovered vessel, viable spores of all three tested species of *Bacillus* spp. were recovered.

Bacillus anthracis Sterne 34F2 [NCTC 8234] ^a Bacillus cereus ATCC 9592 ^b	3 Minutes Boiling	5 Minutes Boiling Uncovered
Bacillus cereus (commercial)	Covered	Viable Spores
Bacillus thuringiensis subsp. israelensis ATCC 35646	No Viable Spores Detected	Viable Spores Detected

^a [NCTC] — the strain currently is listed by the National Collection of Type Cultures, Health Protection Agency, Salisbury, UK.
 ^b ATCC and associated number are registered or nonregistered trademarks of the American Type Culture Collection, Manassas, Virginia, USA.

The average temperatures at boiling, immediately above the water surfaces, were 98.9 °C (210.02 °F) for the covered vessels and 77.3 °C (171.14 °F) for the uncovered vessels. The investigators cautioned that atmospheric pressure and altitude determine the temperature at which water boils and this will affect inactivation conditions. Increasing altitude decreases water's boiling point; increasing barometric pressure increases the boiling point of water.

Following the References, see Supplemental Tables 1s [free available chlorine] and 2s [monochloramine] for the Ct values for all species and strains tested in the seven studies.

Summary Information on the Bacterial Bioterrorism	Agents Used in the Inactivation Studies
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Agent (Disease)	Transmission and Typical Exposure Sources	Geographic Distribution and Natural Hosts	Waterborne Threat?	Persistence
<i>Bacillus anthracis</i> (Anthrax)	Direct person-to-person transmission is rare from skin infections and is not known from inhalation; highly infectious and has high mortality rate; contact with spore-contaminated soils or infected animal by-products such as bone, hair, hide, and under-cooked meat are the major sources of human infection.	<i>B. anthracis</i> can be found worldwide, causing anthrax primarily in grazing mammals such as sheep, cattle, goats, camels, or wild animals such as antelopes and deer. Human anthrax cases are reported from Africa, Asia, Europe, and the Americas, with only a few locations free of any reported disease.	The NRT ^a considers <i>B.</i> <i>anthracis</i> a possible water threat and cautions that re- aerosolization can occur when using spore contaminated water, for example in fire fighting.	<i>B. anthracis</i> spores can remain viable for decades under harsh conditions in the environment and inactivation of spores in biofilms growing in the water treatment system pipes is difficult.
<i>Brucella</i> spp. (Brucellosis)	Direct person-to-person transmission is rare, but has been documented; worldwide, it is the most common disease transmissible to humans from animals and is one of the most common laboratory-acquired diseases; ingesting unpasteurized dairy products or infected animal products is the major exposure route.	Although largely eradicated in much of Europe and North America, brucellosis remains an important human and agricultural health problem in parts of North Africa, the Mediterranean, the Middle East, Asia, India, and Central and South America; can infect a variety of animals, including cattle, sheep, goats, camels, pigs, dogs, reindeer, yaks and many wild mammal species. <i>Brucella</i> spp. or <i>Brucella</i> antibodies have been detected in many marine mammals, including seals, dolphins, porpoises, walruses, and whales.	The NRT considers <i>Brucella</i> spp. a probable water threat because the bacteria are stable in water for 20 to 72 days.	Brucella suis and B. melitensis can persist in soil for up to 125 days. Without exposure to sunlight, under low to moderate temperatures (4 to 22 °C (39 to 72 °F)), B. suis has remained viable for at least 28 days on aluminum, glass, and topsoil.
<i>Burkholderia mallei</i> (Glanders)	Direct person-to-person transmission is rare; infected horses, with and without symptoms, pose the greatest risk for human exposure.	Many countries have eradicated naturally occurring glanders, but it is still found in parts of Africa, the Middle East, Central and South America. Glanders is primarily a disease of horses, mules, and donkeys, but can also be found in other animals.	The NRT reports that <i>B.</i> <i>mallei</i> can survive in water at room temperature in water for one month.	<i>B. mallei</i> can survive in warm, moist environments for a few months. It is not believed to be persistent in soil.

^a NRT – National Response Team, see References: Bioterrorism Agents under "U.S. NRT"

Agent (Disease)	Transmission and Typical Exposure Sources	Geographic Distribution and Natural Hosts	Waterborne Threat?	Persistence
Burkholderia pseudomallei (Melioidosis)	Direct person-to-person transmission is rare, but has been documented; infectivity when aerosolized in not known; people with risk factors such as diabetes or alcoholism are at the greatest risk of contracting severe forms of the disease; melioidosis presents with a wide variety of symptoms in multiple body systems and symptoms can take years to first appear; it can be acute or chronic.	<i>B. pseudomallei</i> is widely found in tropical and subtropical climates, including Southeast Asia, northern Australia, south Asia, and China, as well as sporadically in parts of Africa, Central and South America. Many animal species, including sheep, goats, horses, swine, cattle, kangaroos, camels, dogs and cats, even some species of birds, are susceptible to infection.	The NRT ^a reports that <i>B. pseudomallei</i> is known to persist in water for over three years.	<i>B. pseudomallei</i> is a resilient microbe able to withstand hostile environmental conditions and long periods of nutritional deficiency. It can persist in moist clay soils for up to two years. It can survive in acidic environments (pH 4.5) for 40 days.
Francisella tularensis (Tularemia)	Direct person-to-person transmission is rare from skin and not known from inhalation; highly infectious when aerosolized; can be acquired by direct contact with infected animals, animal bites, ingestion, and inhalation, in addition to being transmitted by arthropods such as deerflies, mosquitos, and ticks.	<i>F. tularensis</i> is found almost entirely in North America and Eurasia. Many animals are susceptible to tularemia, including rabbits and hares, sheep, and many rodents.	The NRT considers water a possible pathway for the weaponized organism. Natural outbreaks of <i>F. tularensis</i> subsp. <i>holarctica</i> in water have occurred.	<i>F. tularensis</i> can persist under cold, moist conditions in hay, water, decaying carcasses, and soil. Live bacteria have been found in rabbit meat after 3 years storage at -15 °C (5 °F).
<i>Yersinia pestis</i> (Plague)	Direct person-to-person transmission of pneumonic plague is possible; human infection is usually from flea bites; the plague has occurred in pandemics as recently as the 1890's (started in China).	<i>Y. pestis</i> is found on all continents except Australia and Antarctica. In the wild, <i>Y. pestis</i> causes disease in over 200 species of rodents as well as rabbits, cats, dogs, and other animals, which can acquire the disease, as well as carry infected fleas	The NRT considers it possible that <i>Y. pestis</i> poses a water threat and reports that it has persisted in spring water under laboratory conditions for 160 days.	Under controlled conditions at 22 °C (72 °F), <i>Y. pestis</i> has remained viable for at least seven days on aluminum and painted dry wall tape; without sunlight, it can remain viable and infectious under controlled conditions in soil for up to 40 weeks.

Summary Information on the Bacterial Bioterrorism Agents Used in the Inactivation Studiescontinued

^a NRT – National Response Team, see References: Bioterrorism Agents under "U.S. NRT"

REFERENCES: Inactivation Studies

[1] O'Connell, H.A., Rose, L.J., Shams, A.M., Arduino, M.J., and Rice, E.W. 2011. <u>Chlorine</u> disinfection of *Francisella tularensis*. *Lett. Appl. Microbiol*. 52(1): 84-86.

[2] O'Connell, H.A., Rose, L.J., Shams, A.M., Bradley, M., Arduino, M.J., and Rice, E.W. 2009. <u>Variability of *Burkholderia pseudomallei* strain sensitivities to chlorine disinfection</u>. *Appl. Environ. Microbiol.* 75(16): 5405-5409.

[3] Rice, E.W., Adcock, N.J., Sivaganesan, M., and Rose, L.J. 2005. <u>Inactivation of spores of</u> *Bacillus anthracis* Sterne, *Bacillus cereus*, and *Bacillus thuringiensis* subsp. *israelensis* by <u>chlorination</u>. *Appl. Environ. Microbiol.* 71(9): 5587-5589.

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Log ₁₀ Reduc-tion	Bacterial Strains Tested	Temperature	➔ At pH 7 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 7 Ct (mg*min/L) at 5 °C (41 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	Bacillus anthracis Ames	25	79	5	220	25	nt ^(a)	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
3 log ₁₀	Bacillus anthracis Ames	25	102	5	339	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
2 log ₁₀	Bacillus anthracis Sterne 34F2	25	60	5	190	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
3 log ₁₀	Bacillus anthracis Sterne 34F2	25	86	5	271	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
2 log ₁₀	Bacillus anthracis Sterne 34F2	23	45	5	140	23	127	5	319	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
3 log ₁₀	Bacillus anthracis Sterne 34F2	23	68	5	210	23	191	5	478	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
4 log ₁₀	Bacillus anthracis Sterne 34F2	23	90	5	280	23	254	5	637	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
2 log ₁₀	Bacillus cereus ATCC 7039 ^(b)	23	41	5	117	23	132	5	340	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
3 log ₁₀	Bacillus cereus ATCC 7039	23	62	5	175	23	199	5	510	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
4 log ₁₀	Bacillus cereus ATCC 7039	23	82	5	233	23	264	5	680	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
2 log ₁₀	Bacillus globigii (Dugway)	23	108	5	372	23	367	5	943	Sivaganesan, M., et al. 2006. J. Water Supply: Res. Technol AQUA. 55(1): 33-43.	[7]
3 log ₁₀	Bacillus globigii (Dugway)	23	136	5	446	23	438	5	1,144	Sivaganesan, M., et al. 2006. J. Water Supply: Res. Technol AQUA. 55(1): 33-43.	[7]
2 log ₁₀	Bacillus thuringiensis subsp. israelensis ATCC 35646	23	66	5	229	23	246	5	481	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
3 log ₁₀	Bacillus thuringiensis subsp. israelensis ATCC 35646	23	99	5	344	23	369	5	721	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]
4 log ₁₀	Bacillus thuringiensis subsp. israelensis ATCC 35646	23	132	5	458	23	492	5	961	Rice, E.W., et al. 2005. Appl. Environ. Microbiol . 71(9): 5587-5589.	[3]

Log ₁₀ Reduc-tion	Bacterial Strains Tested	Temperature	➔ At pH 7 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	➔ At pH 7 Ct (mg*min/L) at 5 °C (41 °F)	Temperature	➔ At pH 8 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	Brucella melitensis ATCC 23456 [NCTC 10094] ^(c)	25	0.1	5	0.3	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
3 log ₁₀	Brucella melitensis ATCC 23456 [NCTC 10094]	25	0.2	5	0.5	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
2 log ₁₀	Brucella suis EAM562	25	0.1	5	0.3	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
3 log ₁₀	Brucella suis EAM562	25	0.2	5	0.4	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
2 log ₁₀	Burkholderia mallei M-13	25	0.1	5	0.2	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
3 log ₁₀	Burkholderia mallei M-13	25	0.2	5	0.2	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
2 log ₁₀	Burkholderia mallei M-9	25	0.1	5	0.2	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
3 log ₁₀	Burkholderia mallei M-9	25	0.2	5	0.2	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
2 log ₁₀	Burkholderia pseudomallei ATCC 1688 [NCTC 1688]	25	0.4	5	0.5	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
3 log ₁₀	Burkholderia pseudomallei ATCC 1688 [NCTC 1688]	25	0.6	5	0.7	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol. 71(1): 566-568.	[6]
2 log ₁₀	Burkholderia pseudomallei ATCC 11668 [NCTC 11642] – source: clinical	25	0.3	5	0.4	25	0.2	5	0.7	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	Burkholderia pseudomallei ATCC 11668 [NCTC 11642] – source: clinical	25	0.5	5	1.1	25	0.4	5	1.3	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	Burkholderia pseudomallei ATCC 11668 [NCTC 11642] – source: clinical	25	0.8	5	1.8	25	0.7	5	1.9	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
2 log ₁₀	Burkholderia pseudomallei ATCC 23343 [NCTC 12939] – source: clinical	25	0.7	5	1.0	25	0.5	5	0.9	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	Burkholderia pseudomallei ATCC 23343 [NCTC 12939] – source: clinical	25	0.9	5	1.4	25	1.1	5	1.9	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	Burkholderia pseudomallei ATCC 23343 [NCTC 12939] – source: clinical	25	1.1	5	1.8	25	1.8	5	2.8	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
2 log ₁₀	Burkholderia pseudomallei AU 631 – source: soil	25	0.1	5	0.1	25	0.1	5	0.2	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	Burkholderia pseudomallei AU 631 – source: soil	25	0.1	5	0.2	25	0.1	5	0.3	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	Burkholderia pseudomallei AU 631 – source: soil	25	0.1	5	0.3	25	0.1	5	0.4	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]

Log ₁₀ Reduc-tion	Bacterial Strains Tested	Temperature	→ At pH 7 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 7 Ct (mg*min/L) at 5 °C (41 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	<i>Burkholderia pseudomallei</i> CA 650 – source: clinical, transiently mucoid [ART] ^(d)	25	0.6	5	0.8	25	1.1	5	1.1	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	<i>Burkholderia pseudomallei</i> CA 650 – source: clinical, transiently mucoid [ART]	25	1.0	5	1.3	25	1.7	5	1.7	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	<i>Burkholderia pseudomallei</i> CA 650 – source: clinical, transiently mucoid [ART]	25	1.5	5	1.7	25	2.3	5	2.9	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
2 log ₁₀	Burkholderia pseudomallei CA 652 – source: clinical, mucoid [ART]	25	0.8	5	2.3	25	0.9	5	3.7	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	Burkholderia pseudomallei CA 652 – source: clinical, mucoid [ART]	25	1.3	5	3.7	25	1.4	5	5.8	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	<i>Burkholderia pseudomallei</i> CA 652 – source: clinical, mucoid [ART]	25	1.7	5	5.0	25	1.9	5	7.8	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
2 log ₁₀	<i>Burkholderia pseudomallei</i> SC 763 – source: clinical, nonmucoid [ART]	25	0.2	5	0.2	25	0.1	5	0.5	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	<i>Burkholderia pseudomallei</i> SC 763 – source: clinical, nonmucoid [ART]	25	0.3	5	0.3	25	0.2	5	0.8	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	Burkholderia pseudomallei SC 763 – source: clinical, nonmucoid [ART]	25	0.4	5	0.5	25	0.3	5	1.1	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
2 log ₁₀	Burkholderia pseudomallei SC 764 – source: clinical, nonmucoid [ART]	25	0.1	5	0.1	25	0.1	5	0.2	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
3 log ₁₀	Burkholderia pseudomallei SC 764 – source: clinical, nonmucoid [ART]	25	0.1	5	0.2	25	0.1	5	0.3	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
4 log ₁₀	Burkholderia pseudomallei SC 764 – source: clinical, nonmucoid [ART]	25	0.1	5	0.3	25	0.2	5	0.5	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]
2 log ₁₀	Burkholderia pseudomallei TH 694 – source: water	25	0.1	5	0.1	25	0.1	5	0.1	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei TH 694 – source: water	25	0.1	5	0.2	25	0.2	5	0.3	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei TH 694 – source: water	25	0.2	5	0.4	25	0.4	5	0.5	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405- 5409.	[2]

Log ₁₀ Reduc-tion	Bacterial Strains Tested	Temperature	→ At pH 7 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 7 Ct (mg*min/L) at 5 °C (41 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	➔ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	<i>Francisella tularensis</i> subsp. <i>holarctica</i> KY99-3387 (type B)	25	0.8	5	14.4	25	2.6	5	33.9	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	<i>Francisella tularensis</i> subsp. <i>holarctica</i> KY99-3387 (type B)	25	1.0	5	18.3	25	3.2	5	43.7	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	<i>Francisella tularensis</i> subsp. <i>holarctica</i> KY99-3387 (type B)	25	1.3	5	22.3	25	3.8	5	53.5	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
2 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	0.6	5	1.5	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
3 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	1.0	5	2.4	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
2 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	0.7	5	5.0	25	2.0	5	15.9	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	1.0	5	6.7	25	2.7	5	20.1	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	1.2	5	8.5	25	3.5	5	24.3	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
2 log ₁₀	Francisella tularensis subsp. tularensis MA00-2987 (A1)	25	0.9	5	13.6	25	2.7	5	64.1	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	Francisella tularensis subsp. tularensis MA00-2987 (A1)	25	1.3	5	16.9	25	3.4	5	83.8	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	Francisella tularensis subsp. tularensis MA00-2987 (A1)	25	1.6	5	20.2	25	4.2	5	103.4	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
2 log ₁₀	Francisella tularensis subsp. tularensis NM99-1823 (A2)	25	0.4	5	14.4	25	2.9	5	45.4	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	Francisella tularensis subsp. tularensis NM99-1823 (A2)	25	0.5	5	17.7	25	3.7	5	60.5	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	Francisella tularensis subsp. tularensis NM99-1823 (A2)	25	0.7	5	21.0	25	4.5	5	75.7	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]

Log ₁₀ Reduc-tion	Bacterial Strains Tested	Temperature	→ At pH 7 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 7 Ct (mg*min/L) at 5 °C (41 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 23 or 25 °C (73 or 77 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	Francisella tularensis subsp. holarctica NY98 (type B)	25	2.0	5	7.8	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
3 log ₁₀	Francisella tularensis subsp. holarctica NY98 (type B)	25	3.9	5	10.3 ^(e)	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
2 log ₁₀	Francisella tularensis subsp. holarctica OR96-0246 (type B)	25	0.9	5	9.3	25	2.7	5	47.1	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	Francisella tularensis subsp. holarctica OR96-0246 (type B)	25	1.2	5	12.9	25	3.7	5	59.0	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	Francisella tularensis subsp. holarctica OR96-0246 (type B)	25	1.5	5	16.5	25	4.6	5	70.8	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
2 log ₁₀	Francisella tularensis subsp. tularensis Schu S4 (A1)	25	0.9	5	13.4	25	3.7	5	47.4	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	Francisella tularensis subsp. tularensis Schu S4 (A1)	25	1.3	5	16.8	25	4.5	5	62.3	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	Francisella tularensis subsp. tularensis Schu S4 (A1)	25	1.7	5	20.3	25	5.2	5	77.2	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
2 log ₁₀	Francisella tularensis subsp. tularensis WY96-3418 (A2)	25	0.8	5	14.2	25	3.3	5	46.8	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
3 log ₁₀	Francisella tularensis subsp. tularensis WY96-3418 (A2)	25	1.3	5	17.4	25	4.1	5	61.7	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
4 log ₁₀	Francisella tularensis subsp. tularensis WY96-3418 (A2)	25	1.6	5	20.8	25	5.0	5	76.2	O'Connell, H.A., et al. 2011. Lett. Appl. Microbiol . 52(1): 84-86	[1]
2 log ₁₀	Yersinia pestis A1122	25	0.4	5	0.5	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
3 log ₁₀	Yersinia pestis A1122	25	0.6	5	0.7	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
2 log ₁₀	<i>Yersinia pestis</i> Harbin	25	0.03	5	0.03	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
3 log ₁₀	Yersinia pestis Harbin	25	0.04	5	0.04	25	nt	5	nt	Rose, L.J., et al. 2005. Appl. Environ. Microbiol . 71(1): 566-568.	[6]
	not tested ^(b) ATCC and the associated number are registerent ency, Salisbury, UK, but no longer listed in the American Type		-		••			-		train currently is listed by National Collection of Type Cultures, Health ed number (see reference 6)	

Log₁₀ Reduc- tion	Bacterial Strains Tested	Temperature	→ At pH 8 Ct (mg*min/L) at 25 °C (77 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 15 °C (59 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	Bacillus anthracis Ames	25	785	15	1,072	5	3,499	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Bacillus anthracis Ames	25	1,204	15	1,691	5	6,813	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Bacillus anthracis Sterne 34F2	25	1,442	15	2,793	5	10,532	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Bacillus anthracis Sterne 34F2	25	1,847	15	3,925	5	15,164	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Brucella melitensis ATCC 23456 ^(a) [NCTC 10094] ^(b)	25	104.4	15	204.0	5	501.8	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Brucella melitensis ATCC 23456 [NCTC 10094]	25	116.6	15	223.9	5	579.5	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Brucella suis MO562	25	47.8	15	99.8	5	134.3	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Brucella suis MO562	25	56.1	15	120.4	5	156.8	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Burkholderia mallei M-9	25	52.5	15	89.4	5	158.6	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Burkholderia mallei M-9	25	64.6	15	102.4	5	194.1	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Burkholderia pseudomallei ATCC 11668 [NCTC 11642] – source: clinical	25	43	15	nt ^(c)	5	204	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei ATCC 11668 [NCTC 11642] – source: clinical	25	49	15	nt	5	238	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei ATCC 11668 [NCTC 11642] – source: clinical	25	54	15	nt	5	262	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
2 log ₁₀	Burkholderia pseudomallei ATCC 23343 [NCTC 12939] – source: clinical	25	49	15	nt	5	190	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei ATCC 23343 [NCTC 12939] – source: clinical	25	73	15	nt	5	226	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei ATCC 23343 [NCTC 12939] – source: clinical	25	97	15	nt	5	251	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]

Log ₁₀ Reduc- tion	Bacterial Strains Tested	Temperature	→ At pH 8 Ct (mg*min/L) at 25 °C (77 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 15 °C (59 °F)	Temperature	→ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	Burkholderia pseudomallei AU 631 – source: soil	25	42	15	nt	5	240	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei AU 631 – source: soil	25	49	15	nt	5	266	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei AU 631 – source: soil	25	55	15	nt	5	291	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
2 log ₁₀	Burkholderia pseudomallei CA 650 – source: clinical, transiently mucoid [ART] ^(d)	25	50	15	nt	5	138	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei CA 650 – source: clinical, transiently mucoid [ART]	25	68	15	nt	5	202	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei CA 650 – source: clinical, transiently mucoid [ART]	25	86	15	nt	5	266	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
2 log ₁₀	Burkholderia pseudomallei CA 652 – source: clinical, mucoid [ART]	25	70	15	nt	5	234	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei CA 652 – source: clinical, mucoid [ART]	25	88	15	nt	5	281	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei CA 652 – source: clinical, mucoid [ART]	25	99	15	nt	5	328	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
2 log ₁₀	Burkholderia pseudomallei KC 872	25	38.8	15	87.6	5	116.7	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Burkholderia pseudomallei KC 872	25	45.9	15	103.9	5	156.1	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Burkholderia pseudomallei SC 763 – source: clinical, nonmucoid [ART]	25	53	15	nt	5	302	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei SC 763 – source: clinical, nonmucoid [ART]	25	60	15	nt	5	382	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei SC 763 – source: clinical, nonmucoid [ART]	25	68	15	nt	5	462	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
2 log ₁₀	Burkholderia pseudomallei SC 764 – source: clinical, nonmucoid [ART]	25	48	15	nt	5	266	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei SC 764 – source: clinical, nonmucoid [ART]	25	56	15	nt	5	288	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei SC 764 – source: clinical, nonmucoid [ART]	25	65	15	nt	5	310	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]

Log₁₀ Reduc- tion	Bacterial Strains Tested	Temperature	➔ At pH 8 Ct (mg*min/L) at 25 °C (77 °F)	Temperature	➔ At pH 8 Ct (mg*min/L) at 15 °C (59 °F)	Temperature	➔ At pH 8 Ct (mg*min/L) at 5 °C (41 °F)	Citations	Reference Number
2 log ₁₀	Burkholderia pseudomallei TH 694 - source: water	25	99	15	nt	5	404	O'Connell, H.A., et al. 2009. Appl. Environ. Microbiol. 75(16): 5405-5409.	[2]
3 log ₁₀	Burkholderia pseudomallei TH 694 - source: water	25	113	15	nt	5	477	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
4 log ₁₀	Burkholderia pseudomallei TH 694 - source: water	25	127	15	nt	5	550	O'Connell, H.A., et al. 2009. <i>Appl. Environ. Microbiol.</i> 75(16): 5405-5409.	[2]
2 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	26.3	15	61.2	5	76.0	Rose, L.J., et al. 2007. <i>Appl. Environ. Microbiol</i> . 73(10): 3437- 3439.	[5]
3 log ₁₀	Francisella tularensis subsp. holarctica LVS (type B)	25	30.4	15	71.1	5	97.9	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Francisella tularensis subsp. holarctica NY98 (type B)	25	31.3	15	48.7	5	84.0	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Francisella tularensis subsp. holarctica NY98 (type B)	25	37.1	15	64.8	5	116.0	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
2 log ₁₀	Yersinia pestis A1122	25	27.6	15	71.4	5	92.0	Rose, L.J., et al. 2007. <i>Appl. Environ. Microbiol</i> . 73(10): 3437- 3439.	[5]
3 log ₁₀	Yersinia pestis A1122	25	33.1	15	86.4	5	115.6	Rose, L.J., et al. 2007. <i>Appl. Environ. Microbiol</i> . 73(10): 3437- 3439.	[5]
2 log ₁₀	Yersinia pestis Harbin	25	21.9	15	33.5	5	80.7	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]
3 log ₁₀	Yersinia pestis Harbin	25	25.0	15	40.8	5	91.4	Rose, L.J., et al. 2007. Appl. Environ. Microbiol . 73(10): 3437- 3439.	[5]

KEY^(a) ATCC and the associated number are registered or nonregistered trademarks of the American Type Culture Collection, Manassas, Virginia, USA^(b) [NCTC] – the strain currently is listed by National Collection of Type Cultures, Health Protection Agency, Salisbury, UK, but no longer listed in the American Type Culture Collection ^(c) nt = not tested^(d) ART – source is the "Antimicrobials Resistance Team, CDC"