

1 Use of Household Bleach Products for Emergency Disinfection

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Abstract

29 Household bleach is typically used as a disinfectant for water in times of emergencies
30 and by those engaging in recreational activities such as camping, hiking or rafting. The
31 Centers for Disease Control and Prevention recommends a concentration of free
32 chlorine of 1 mg/L for 30 minutes, or about 0.75 mL (1/8 teaspoon) of household bleach
33 per gallon of water. A new non-staining taste-less bleach product has recently become
34 available for household use, which some individuals may prefer over standard bleach
35 products. This recommended concentration of free chlorine is achieved by the addition
36 of 50 mL (approximately ¼ cup) of this product per gallon of water. This product was
37 found to be equally as effective as standard household bleach, however required a
38 larger volume to achieve the level of log reduction. Both products meet the
39 requirements of the United States Environmental Protection Agency (USEPA) test
40 protocol for individual water treatment for the reduction of *Klebsiella terrigena*, but not
41 for polio virus type I.

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Introduction

43 Surface waters can be expected to be contaminated from time to time by fecal matter
44 from animals and man. Many people such as military personnel, campers, hikers and
45 rafters that use water from rivers, lakes and other surface water sources that may be
46 contaminated are at risk of becoming ill from enteric pathogens excreted in the feces.
47 Natural disasters such as hurricanes, floods and earthquakes can also cause
48 contamination of drinking water. Drinking water contamination can also be a problem for
49 visitors to foreign countries where treatment and distribution deficiencies may be

50 common. Disinfection in these situations to reduce the risk of illness from waterborne,
51 disease-causing microorganisms is necessary.

52 Currently, the Centers for Disease Control and Prevention (CDC) recommends that
53 household bleach be used as a drinking water disinfectant (for bacteria and viruses) in
54 emergency situations by adding 0.75 mL (1/8 teaspoon) to one gallon of water and
55 letting it stand for 30 minutes before use. This yields an effective free chlorine
56 concentration in the water of approximately 1 mg/L. However, household bleach can
57 damage and stain sensitive materials, and has a detectable taste or odor to the average
58 person. Recently a non-staining, odor and tasteless (to the average person) bleach
59 product has been developed, which is available to the consumer for household use.

60 Since it overcomes some of the objectives with the use of regular household bleach, its
61 efficacy as a drinking water disinfectant was assessed following the requirements of the
62 United States Environmental Protection Agency (USEPA) test protocol for individual
63 water treatment.

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Materials and Methods

66 The general test procedures for individual water treatment as outlined in the “Guide
67 Standard and Protocol for Testing Microbiological Water Purifiers” were used in this
68 study (USEPA, 1987).

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70 *Preparation of test waters:*

71 Approximately three gallons of dechlorinated Tucson tap water (Table 1) was collected
72 in a 6-gallon plastic carboy. Tucson tap water is obtained from deep aquifers. The pH

73 was adjusted with 1 N NaOH to the desired test pH and the turbidity adjusted according
74 to the desired test turbidity of 30 NTU using AC spark plug (General Motors Corp., Flint
75 Michigan). Humic acid (Aldrich Chemical Company, Inc., Milwaukee, WI) was added to
76 obtain an organic carbon concentration of 10 mg/L. Two types of test water are used
77 referred to as average and worst case water conditions (USEPA, 1987). Table 1 shows
78 the composition of average and worst case water.

79 *Preparation of bacteria:*

80 *Klebsiella terrigena* (ATTC 33257) was obtained from the American Type Culture
81 Collection (Bethesda, MD). *Klebsiella terrigena* was grown as an overnight culture at
82 37°C in trypticase soy broth (Becton, Dickinson and Company, Sparks, MD). In order to
83 remove the growth media, the bacterial culture was pelleted by centrifugation and
84 resuspended in 0.01M phosphate buffered solution (PBS) three times.

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86 *Preparation of virus:*

87 Poliovirus type 1 (strain LSc-2ab) was obtained from the culture collection of the
88 Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX.
89 Poliovirus type 1 was propagated using the buffalo green monkey (BGM) cell line. A
90 freeze thaw cycle was performed three times (to release the virus from the infected
91 cells) by freezing at -20°C and thawing at 37°C. Following the freeze thaw, the cell
92 culture fluid was subject to concentration with polyethylene glycol extraction (Thurston-
93 Enriquez et al., 2003). The virus concentrate was then stirred overnight at 4°C,
94 centrifuged at 4°C for 30 minutes at 8,000 rpm and resuspended in 10% 0.01 M PBS. A

95 final Vertrel XF (Micro Care Corp, New Britain, CT) extraction was done to reduce
96 dissolved organics. The virus was stored at -80°C until needed.

97 *Experimental Procedure:*

98 Following the adjustment of the test water to the appropriate test conditions, 3.78 liters
99 was aliquoted to one of three designated 4L plastic beakers. One beaker was used as a
100 control (no disinfectant), one beaker was dosed with 1 mg/L (0.75 mL) household
101 bleach and the third beaker was dosed with 1 mg/L (50 mL) Clorox Anywhere®. The
102 same chlorine concentrations were used for both *K. terrigena* and poliovirus type 1.
103 Each beaker was inoculated with approximately 1.0×10^7 colony forming units (cfu)/mL
104 of *Klebsiella terrigena*. For the reduction of virus, each beaker was inoculated with $1.0 \times$
105 10^5 plaque forming units (pfu/mL) of poliovirus type 1. Prior to adding the disinfectant, a
106 sample was collected after bacterial or virus addition to determine the initial
107 concentration (T_0) of the organism. Samples were immediately placed in sterile tubes
108 containing 1 mL of 10% sodium thiosulfate. Virus samples were collected at 1, 10 and
109 30 minutes. Chlorine residual was measured when each sample was collected using
110 Standard Method #4500 (APHA, 2005) and a HACH DR 2000 spectrophotometer
111 (Loveland, CO). The control beaker contained only bacteria or virus but no disinfectant.
112 Samples from the control beaker were collected at the beginning of the experiment (T_0)
113 and after 30 minutes (T_{30}) and placed in a dilution tube containing 1 mL 10% sodium
114 thiosulfate. Chlorine residual was also measured at each time a sample was collected
115 for the control. Bacterial samples were stored at 4°C. All bacterial samples were
116 assayed in duplicate by Standard Method 9215 on mEndo media (Becton, Dickinson,
117 and Company, Sparks, MD) (APHA 2005).

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119 Virus samples were stored in 5 mL aliquots at -20°C and subsequently thawed for
120 enumeration. The virus samples were thawed at room temperature and filtered using
121 sterile 0.22 µm pore size Acrodisc syringe filters pretreated with 3% beef extract, pH
122 7.00 (Pall, Ann Arbor, MI, USA) to remove bacteria. Samples were assayed in 6-well
123 plastic plates containing BGM cells in volumes of 100 µL in duplicate. They were then
124 overlaid with agar containing Eagle's Minimal essential media containing 2% fetal
125 bovine serum. The samples were incubated for 48 hours at 37°C with 5% CO₂, the agar
126 overlays were removed and the cell monolayers stained with crystal violet and viral
127 plaques counted.

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Results

130 The inactivation of the two test organisms under general water quality conditions are
131 shown in Tables 2 and 3. As expected *K. terrigena* was far more sensitive to
132 inactivation than the poliovirus type 1, being reduced to undetectable levels in less than
133 30 minutes under all test water conditions. The needed 4 log₁₀ reduction of poliovirus
134 type 1 was not achieved in any of the test water conditions. Only a 3.45 log₁₀ reduction
135 was achieved at room temperature and near neutral pH conditions for poliovirus type 1
136 with both household bleach and the alternative bleach product.

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Discussion

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The United States Environmental Protection Agency (USEPA) requires that
personal drinking water treatment be capable of killing/inactivating 99.9999% (6 log₁₀)

143 *Klebsiella terrigena*, and 99.99% (4 log₁₀) of poliovirus type 1 to ensure its
144 microbiological safety. The disinfectant must achieve these levels of inactivation at both
145 pH 7.5 and pH 9.0 in clear and turbid water containing a heavy organic load (USEPA,
146 1987). Filtration of the water, in addition to disinfection is recommended if
147 *Cryptosporidium* oocysts are suspected to be present.

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149 Overall, the performance of household bleach and the alternative product being tested,
150 Clorox Anywhere[®], appeared to be equally effective against *K. terrigena* in the various
151 test waters. Both were capable of killing 6 log₁₀ of *K. terrigena* in less than one minute
152 in low and high turbidity water at pH 7.5 even in the presence of a heavy organic load.
153 Only at pH 9.0 was the time increased from one to five minutes to achieve the same
154 level of effectiveness. The effectiveness of the Clorox Anywhere[®] and the household
155 bleach was much lower when poliovirus type 1 was used. There was approximately
156 over a 3 log₁₀ reduction of poliovirus in water with conditions of pH 7.5, 25°C and no
157 turbidity after one minute for both the Clorox Anywhere[®] and household bleach. With the
158 conditions of pH 9.0, 30 NTU and 5°C both the Clorox Anywhere[®] and household bleach
159 resulted in a 1 log₁₀ reduction of poliovirus. There was a 0.5 log₁₀ reduction of poliovirus
160 with both Clorox Anywhere[®] and household bleach when the conditions of pH 7.5, 25°C
161 and 30 NTU were used. Both the Clorox Anywhere[®] and household bleach were equally
162 effective against *K. terrigena* and poliovirus type 1, however a larger volume of Clorox
163 Anywhere is needed compared to bleach due to its lower concentration of hypochlorite.
164 An advantage of Clorox Anywhere[®] is that it has no odor or taste compared to

165 household bleach. It is also not corrosive like bleach and can be handled with bare
166 hands.

167 Organic matter has been found to protect viruses from degradation. This may be due to
168 the fact that a virus can adsorb to organic matter, which makes inactivation more
169 difficult. This may be why the \log_{10} reduction of polio virus type I is much lower for both
170 Clorox Anywhere and household bleach in the worst case water conditions. As far as
171 pH is concerned, it seemed that in highly turbid conditions (worst case water), pH
172 changes did not have a significant changes in \log_{10} reduction for household bleach,
173 however the same is not true of Clorox anywhere. In highly turbid conditions, at a higher
174 pH Clorox Anywhere seems to be more effective, with a larger \log_{10} reduction value. In
175 the worst case water conditions, there is high turbidity and thus more organic material in
176 the water which increases the chlorine demand. Therefore water in this condition may
177 require higher concentrations of chlorine which could be why polio virus type I did not
178 achieve the required 4 \log_{10} reduction.

179 The results suggest that the current recommendations for treatment of drinking water in
180 emergency situations cannot meet the recommendation for enteric viruses by the United
181 States Environmental Protection Agency. However, when the water is treated at near
182 room temperature and near neutral pH the \log_{10} reduction needed is very close to the
183 recommended levels for safe drinking water. Caution should be used in treatment of
184 water with high turbidity and pH.

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References

American Public Health Association (APHA). 2005 L.S. Clesceri, A.E. Greenberg, and A.D. Eaton (ed.) Standard Methods for the Examination of Water and Wastewater 21st ed. APHA, Washington, D.C.

Thurston-Enriquez, J. A., C. N. Haas, and J. Jacangelo and C. P. Gerba. 2003. Chlorine inactivation of adenovirus type 40 and feline calicivirus. Appl. Environ. Microbiol. 69:3979-3985.

USEPA. 1987. United States Environmental Protection Agency. Guide Standard and Protocol for Microbiological Water Purifiers. Washington, DC.

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Table 1. Test Waters used in Microbiological Challenges*

Water Type	Turbidity (NTU)	pH	Total organic carbon (mg/L)
Average case	<0.50	7.5	<1.0
Worst case	30	9.0 and 5.0	10

229 *From: USEPA, 1987

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Table 2. Inactivation of *Klebsiella terrigena* and Poliovirus by Clorox Anywhere® (log₁₀ reduction after 30 min)

Organism	pH 7.5, 25°C	pH 9.0, 25°C	pH 7.5, 25°C, 30 NTU	pH 9.0, 25°C, 30 NTU	pH 9.0, 5°C, 30 NTU
Poliovirus	3.45	ND	0.4261	0.7000	0.8536
<i>K. terrigena</i>	>6.45	>6.11	>6.21	>6.10	>5.72

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236 *ND- Not done

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Table 3. Inactivation of *Klebsiella terrigena* and Poliovirus by Household Bleach. (reduction (log₁₀) after 30 min)

Organism	pH 7.5, 25°C	pH 9.0, 25°C	pH 7.5, 25°C, 30 NTU	pH 9.0, 25°C, 30 NTU	pH 9.0, 5°C, 30 NTU
Poliovirus	3.45	ND	0.5982	0.4771	0.5499
<i>K. terrigena</i>	>6.26	>5.94	>6.02	>5.99	>6.11

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244 *ND- Not done

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