NCDENR

North Carolina Department of Environment and Natural Resources

Dexter R. Matthews, Director

Division of Waste Management

Michael F. Easley, Governor
William G. Ross Jr., Secretary

September 7, 2006

Donii Fox, MSPH, CIH
Biological Safety Officer
University of North Carolina at Chapel
Department of Environment, Health, Safety
212 Finley Golf Course Rd.
Campus Box 1650
Chapel Hill, NC 27517-4440

Dear Donii Fox:

This is in response to your letter requesting approval of the chemical treatment of HIV cultures using a 10% bleach solution.

According to 15A NCAC 13B .1207(4)(b) the Division is authorized to approve the alternative chemical treatments of microbiological wastes.

The chemical treatment of HIV contaminated waste as described in the revised procedures for treatment of such waste, which were submitted with your letter of August 30, 2006, is approved.

The publications and other documents that were submitted to the Department substantiate the efficacy of the inactivation of HIV virus with a 10% bleach solution.

Should you have any questions regarding this matter you may contact me at (919) 508-8499 or Bill Patrakis at (919) 508- 8512.

Sincerely,

Ellen Lorscheider

Ellen Lorscheider
Environmental Programs Manager

Cc: Bill Patrakis, Environmental Biologist

Request for approval must be substantiated by results of demonstrated effectiveness of the chemical to treat the specific microbiological agent(s) of concern for the waste disposed.

I. Description of infectious waste

a. *Describe waste to be treated (i.e. cultures, cell lines):* Human blood and other body fluids, HIV cultures, saline solutions used to wash cells
b. *Organisms present*: HIV-1
c. *Estimated concentration/titer of organisms*: $\leq \log 10^5$
d. *Other material present in waste (i.e. other organic material)*: fetal calf serum
e. *Volume of waste and frequency*: 10 ml of blood, 10 ml HIV culture fluid, 500 ml wash buffer from cell isolation procedure daily

II. Description of treatment procedures

a. *Summarize proposed procedure for treating waste:* All work is conducted in a biological safety cabinet to protect the specimens from outside contamination and to contain infectious agents in case of a spill. 90% of the waste consists of wash solutions from peripheral blood mononuclear cell isolations and contains little, if any, HIV. Some blood and cell culture fluid (< 10 ml total volume) remaining in pipettes are also disposed of in the waste container. This liquid waste from specimen processing or HIV cultures is either pipetted or poured into a 2000 mL Nalgene beaker containing 200 mL of 10% (v/v) household bleach. Waste remains in the discard container for a minimum of 1 hour at ambient temperature and up to 6 hours.
b. *Disinfectant to be used (please attach MSDS)*: Ultra Clorox regular bleach = 6.15% sodium hypochlorite (Appendix A)
c. *Disinfectant concentration*: Starting material is 10% ultra Clorox regular bleach = 0.615% sodium hypochlorite = 6150 ppm; Final concentration is approximately 5% household bleach = 0.307% sodium hypochlorite = 3075 ppm
d. *Ratio of disinfectant (ml) to liquid waste (ml)*: Bleach to waste = 1:1
e. *Contact time of disinfectant with liquid waste prior to disposal:* 1-6 hours at ambient temperature (22-25C)
f. *Small variations in temperature, time, pH, concentration and state of dispersion, penetrability, reactivity of organic material may make large differences in the effectiveness of disinfection. List the factors that may affect disinfection*: Temperature is fairly constant at 22-25C. Time is always at least 2 hours. No solids are placed in the waste container and liquids are well dispersed. Very little organic material is present compared with the volume of bleach and wash buffers. The organic material consists of small amounts of human plasma and fetal bovine serum.

III. Verification of efficacy of treatment procedures

a. *Submit results of experiments that verify the proposed procedures are effective. Such studies may include attempts to recover and quantitate the agent from liquid or swab samples, or sealed patches, by animal inoculation, plaque assay, agar or broth cultivation and similar methods, following controlled decontamination under the same experimental conditions envisioned for proposed studies. Reports of these studies should be provided with this document in support of your request.*

Sattar and Springer (attached) reviewed the literature describing inactivation of HIV and stated "Quantitative recovery of infectious HIV or other enveloped viruses from whole blood after disinfection is difficult or impossible. Therefore, studies of disinfectant efficacy against enveloped viruses in whole blood can be undertaken only if a suitable animal model is available."

Our method dilutes blood and culture material by at least 1:50 in a mixture of bleach, detergent, and saline. Cell cultures cannot be used for these purposes since the bleach, the water, and the detergent individually as well as in tandem would kill any cells used as indicators. Simply diluting the starting material by that much would make it next to impossible to obtain a positive culture. The only animal model for HIV is the *Rhesus macaque* model. I do not believe any Institutional Animal Care and Use Committee (IACUC) would approve the use of these expensive and limited animals for this use.

The only practical way to perform these experiments is to take concentrated virus, treat with the disinfectant for a given period of time, neutralize the disinfectant, dilute in culture medium and test in a culture system. Although we have not specifically done these experiments, Dr. David Weber and colleagues of the UNC Division of Infectious Diseases have (1999, Appendix B), using HSV-1 as a surrogate for HIV-1. They found that 1:10 bleach (5000 ppm), even in the presence of 80% blood, could inactive > 4 log of HSV within 1 minute. A 1:100 dilution of bleach in the absence of blood reduced infectivity by > 4 log within 1 minute. Although 1:100 bleach could not completely inactive HSV-1 in the presence of 80% blood, that does not describe the situation used in our experiments where there is little organic material and a lot of saline. In addition we include 1% detergent in the waste container which will further inactive HIV-1.

The Centers for Disease Control and Prevention recommend the use of 1:10-1:100 dilutions of household bleach for the prevention of transmission of HIV in health care settings (1995) (attached, Appendix C), and warn that repeated exposure to sodium hypochlorite is corrosive.

b. *Please attach any publications that will support the use of this disinfectant under the proposed conditions. These publications <u>cannot</u> be provided in lieu of the experiments described above unless the publication describes the same treatment procedures for the infectious waste described in Section I (including concentration of organism, organic material present, type of waste, organisms).*

In addition to the paper by Weber, et al., and the CDC recommendation, I am attaching 5 other relevant papers that describe inactivation of HIV with household bleach. The most comprehensive is a critical review of the literature by Sattar and Springthorpe (1991, Appendix D). They state "The general conclusion from most of these studies is that HIV is a relatively fragile virus that behaves much like any other enveloped virus when challenged with disinfectants." They recommend that "the chemicals most often recommended for the inactivation of HIV are sodium hypochlorite (0.5-1.0%) and glutaradldehyde (2%); some HIV research workers routinely use 5-6% hypochlorite which is corrosive and produces toxic fumes. Such high concentrations may be necessary only for large blood spills."

Martin et al, (1985, Appendix E) found that the minimal concentration of household bleach that completely inactivated $10^{5.24}$ log of infectious HIV incubated for 2-10 minutes at room temperature was 0.1% (52.5 ppm). The expected log reduction in infectivity calculated by these investigators was 1,402.

Similarly, Resnick et al. (1986, Appendix F) found that a 1:10 dilution of household bleach completely inactivated highly concentrated HIV culture supernatants diluted in 50% human plasma within one minute, a reduction of ≥ 7 $\log_{10}$ tissue culture infectious

$dose_{50}$. These levels of concentration are several orders of magnitude higher than are typically seen in patient samples or even in routine HIV cultures.

Bloomfield et al. (1990, Appendix G) found that as little as 50 ppm of sodium hypochlorite was sufficient to completely inactivate 2×10^4 infectious HIV virions in 2 minutes. In the presence of 10% or 50% plasma or blood it took 2500 ppm or 5000 ppm to completely inactivate 2×10^5 infectious virions in 2 minutes. The authors suggest that 3-4 log of HIV can be inactivated within a 2 minute contact with 50 ppm of sodium hypochlorite.

Aranda-Anzaldo, et al (1992, Appendix H) concluded that "Of all the products tested, Triton X-100 and sodium hypochlorite show the strongest virucidal activity, being able to inactivate very high concentrations of HIV even after short incubations (1 min), suggesting that these compounds are the most reliable for disinfection of HIV-contaminated material or surfaces."

Appendices

A. MSDS Sheet – Ultra Clorox regular bleach
B. Weber DJ, Barbee SL, Sobsey MD, Rutala WA. 1999. The effect of blood on the antiviral activity of sodium hypochlorite, a phenolic, and a quarternary ammonium compound. Infection Control and Hospital Epidemiology 20:821-827.
C. Centers for Disease Control and Prevention. 1995. Use of bleach in prevention of transmission of HIV in health care settings. www.cdc.gov/od/ohs/biosfty/bleachhiv.html
D. Sattar SA and Springthorpe VS. 1991/ Survival and disinfectant inactivation of the human immunodeficiency virus: a critical review. Rev Infectious Dis 13:430-47.
E. Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/Lymphadenopathy-associated virus. J Infect Dis 152:400-403.
F. Resnick L, Veren K, Salahuddin SZ, Tondreau S, Markham PD. 1986. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA 255: 1887-1891.
G. Bloomfield SF, Smith-Burchenll CA, Dalgleich AG. 1990. Evaluation of hypochlorite-releasing disinfectants against the human immunodeficiency virus (HIV). J Hosp Infection 15:273-278.
H. Aranda-Anzaldo A, Viza D, Busnel RG. 1992. Chemical inactivation of human immunodeficiency virus in vitro. J Virol Methods 37:71-82.