



North Carolina Department of Environment and Natural Resources

Beverly Eaves Perdue
Governor

Division of Waste Management
Dexter Matthews
Director

Dee Freeman
Secretary

May 8, 2009

Darren Tremi, MS, CHES
Associate Biological Safety Officer
University of North Carolina at Chapel
Department of Environment, Health, Safety
1120 Estes Drive Extension
Campus Box 1650
Chapel Hill, NC 27599-1650

Dear Darren Tremi:

This is in response to your letter requesting approval of the chemical treatment of *coronavirus tissue culture* using 10% sodium hypochlorite (bleach) solution as described in the request for approval submitted to the Department.

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 the organisms listed above as described in the procedures for treatment which was submitted with your letter of April 17, 2009, is approved.

The test descriptions and results which were submitted to the Department substantiate the efficacy of the treatment of the organisms with 10% bleach solution.

Should you have any questions regarding this matter you may contact me at (919) 508-8499 or Bill Patrakis at (336) 771- 5091.

Sincerely,

Ellen B. Lorscheider
NCDENR DWM SWS
2009.05.08 11:03:06
-04'00"

Ellen Lorscheider
Environmental Programs Manager

Cc: Bill Patrakis, Environmental Biologist



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Request for Approval

Chemical Treatment of Liquid Infectious Waste

Approval for chemical treatment of liquid infectious waste must be obtained from the NC Division of Waste Management. Please provide answers to the following questions, attach supporting documents as outlined below, and submit your request to Darren Trembl, EHS, CB #1650. EHS will submit your request to the NC Division of Waste Management.

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 infected:

Mammalian tissue culture cells, supernatant from infected mammalian cells, infected primary human or primate airway epithelial cell cultures

- b. Organisms present:

Human coronaviruses (NL63, OC43, 229E, HKU1), murine coronavirus (mouse hepatitis virus), porcine coronavirus (transmissible gastroenteritis virus)

- c. Estimated concentration/titers of organism:

Titers can range from 10^4 to 10^8 plaque forming units per mL of stock viruses

- d. Other material present in the waste:

Mammalian tissue culture cell lysate (cells lysed or killed by virus infection), synthetic bovine serum for media (Fetal Clone II Hyclone)

- e. Volume and waste frequency:

Total of 50mL of waste per week for the entire lab (includes all experiments, not the volume for a single experiment)



II. Description of treatment procedures

- a. Summarize proposed procedure for treating waste:

All coronavirus infections are performed under a BSL2 certified biosafety cabinet and all waste will be disinfected prior to removal from the cabinet. All waste will be immediately placed into a greater than or equal to 10% bleach/90% water containing solution. Waste will be treated with bleach solution for at least 2 hours prior to disposal.

- b. Disinfectant to be used (please attach MSDS)

10% solution of Ultra Chlorox regular bleach= 6.15% sodium hypochlorite (see appendix materials for MSDS)

- c. Disinfectant concentration:

Starting material is at least 10% Ultra Chlorox regular bleach=0.615% sodium hypochlorite=6150ppm; Final concentration is approximately 5% household bleach= 0.307% sodium hypochlorite

- d. Ratio of disinfectant (mL) to liquid waste (mL):

10ml of waste to 100mL 10% bleach

- e. Contact time of disinfectant with liquid waste prior to disposal:

At least 2 hours at ambient temperature (22-25C) prior to disposal

- 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:

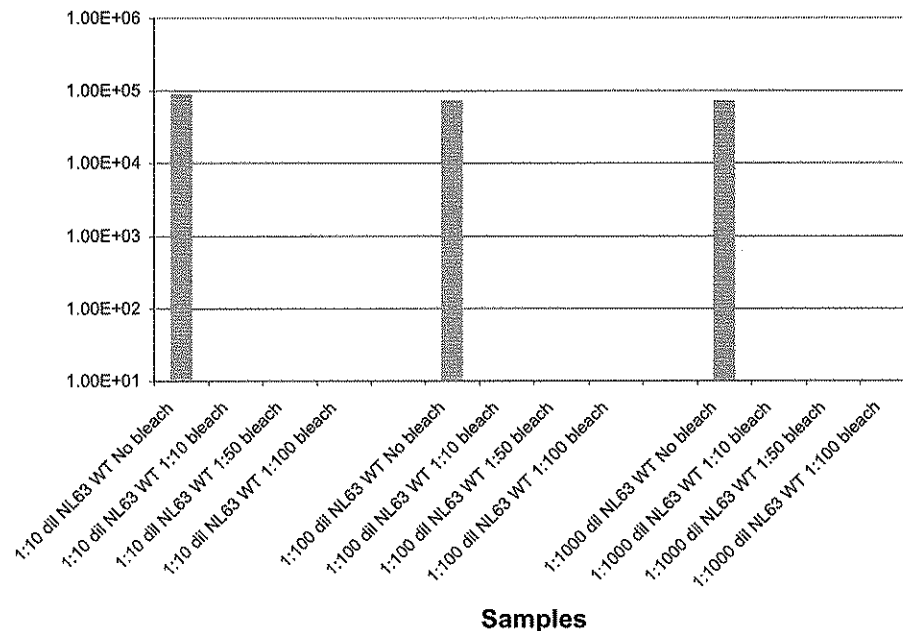
Temperature is consistently between 22-25C. Time is always at least 2 hours. No solids are placed in the waste container and liquids are well dispersed. Very little organic material is present compared with the volume of bleach and tissue culture waste. The organic material consists of small numbers of lysed cells and synthetic fetal bovine serum.

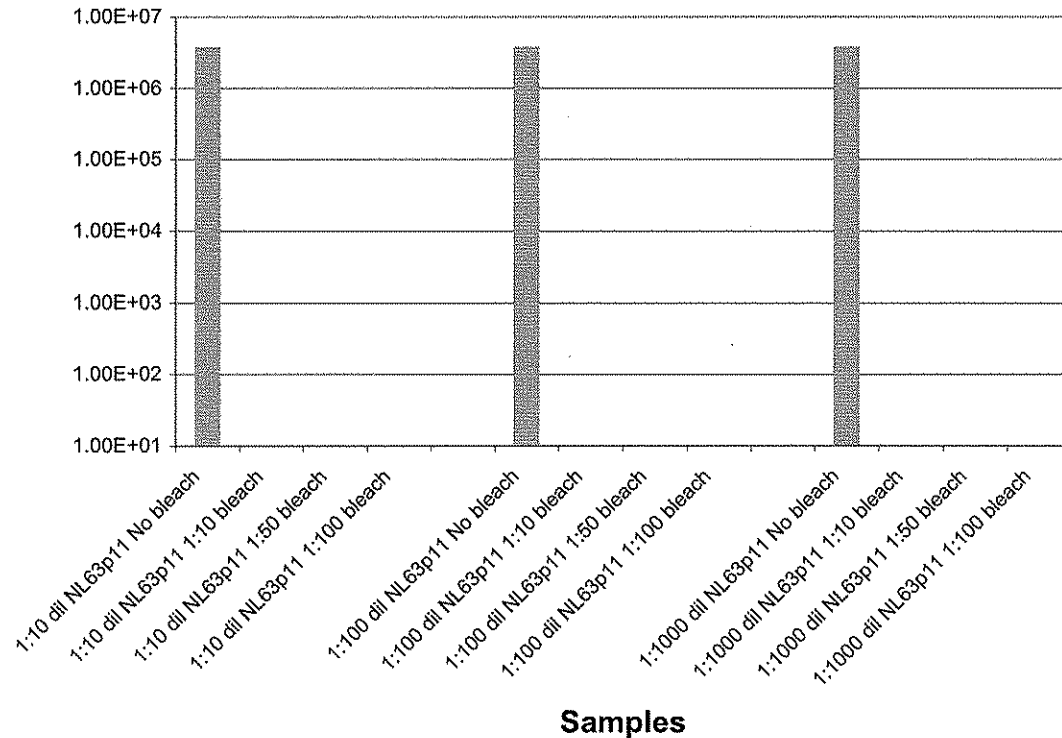


III. Verification of efficacy of treatment procedures

- a. Submit results of experiments that verify the proposed procedures are effective. Such studies may include attempts to recover and quantitate the agent in 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.

Stocks of the BSL2 human coronavirus, either the wild type or growth adapted passage mutant p11 of NL63, were diluted 1:10, 1:100, or 1:1000. The diluted samples were then either mock treated or treated with the following dilutions of bleach: 1:10, 1:50, or 1:100 at room temperature for 2 hours. Mock-treated and bleach treated samples were assayed by viral plaque assay on LLCMK2 cells. The results of the viral titering are presented below. No virus was detected with any samples that were treated with bleach.





- b. Please attach any publications that will support the use of this disinfectant under the proposed conditions. These publications cannot 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 organisms, organic material present, type of waste, organisms).

The following publication is attached:

Lai MY, Cheng PK, Lim WW. 2005. Survival of Severe Acute Respiratory Syndrome Coronavirus. *Clinical Infectious Diseases* 41:e67-71.

Summary:

Lai et al demonstrate that serial dilutions of the BSL3 human coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV), are inactivated by bleach in as little as 5 minutes at room temperature.