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 10, 2008

Darren Treml, 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 Treml:

This is in response to your letter requesting approval of the chemical treatment of *Adenoviral 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 August 19th, 2008, 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 Lorscheider

Ellen Lorscheider
Environmental Programs Manager

Cc: Bill Patrakis, Environmental Biologist

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

ENVIRONMENT, HEALTH & SAFETY

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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 Donii Fox, 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 (i.e. cultures, cell lines):

   Liquid tissue culture waste collected in 5L vacuum flasks generated from Human Bone Osteosarcoma Epithelial Cells (U2OS Line) infected with Ad-GFP adenovirus ranging in titer from 10^8 - 10^{10} pfu/mL.

   b. Organisms present:

   Adenovirus of Ad-GFP.

   c. Estimated concentration/titer of organisms:

   10^8 - 10^{10} pfu/mL

   d. Other material present in waste (i.e. other organic material):

   DMEM (Dulbecco's Modified Eagle's Medium)

   e. Volume of waste and frequency:

   1.0L monthly

II. Description of treatment procedures
   a. Summarize proposed procedure for treating waste:

   Treat vacuum flask waste with a volume of bleach equal to 10% of the total waste volume for 30 minutes prior to disposal to the drain.

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

   Sodium hypochlorite (bleach)

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c. Disinfectant concentration:

10% dilution of total volume of waste.

d. Ratio of disinfectant (ml) to liquid waste (ml):

100mL bleach to 900mL liquid waste collected in double HEPA-barrier vacuum flask.

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

30 minutes

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:

No known factors affecting disinfection.

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.

Attached: *Confirming that proposed 10% bleach treatment of adenoviral tissue culture waste is effective.*

UNC-CH
22-073 Lineberger Comprehensive Cancer Center
Lab of Yue Xiong, PhD
Matthew Smith, Lab Manager

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 organism, organic material present, type of waste, organisms).

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Attached: D. Spector, R. Goldman, L. Leinwand, "Use and Application of Adenovirus Expression Vectors", *Cells: A Laboratory Manual.* Chapter 90. pp. 90.1-90.5.

Contained in this peer reviewed manual (listed on 90.5) are the recommended and acceptable disposal instructions for liquid waste media (without any additional organics generated) from cell lines infected with the same concentration (or more) of adenovirus.