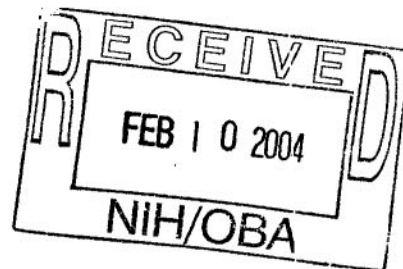


National
Cancer
Institute
At
Frederick



NCI-Frederick Institutional Biosafety Committee
P.O. Box B
Frederick, MD 21702-1201

February 9, 2004



Dr. Eugene Rosenthal
Biotechnology Program, Office of Biotechnology Activities
National Institutes of Health
6705 Rockledge Drive, Suite 750, MSC 7985
Bethesda, MD 20892-7985

Dear Dr. Rosenthal:

This memorandum is in reference to a conversation that the NCI-Frederick Biosafety Officer, Mr. Joseph Kozlovac, had with Alan Shipp on February 4, 2004. The conversation was regarding a centrifuge accident involving a *Herpes simplex* virus derivative rRp450 that is defective in the expression of viral ribonucleotide reductase (ICP6). This accident occurred on November 14, 2003 and was reported to the NCI-Frederick IBC on November 18, 2003. The NCI-Frederick Contract Officer has also been informed of the accident in accordance with Article C.2.a.(7) of NCI Contract N01-CO-12400.

The accident did not result in a laboratory acquired infection and no significant damage to equipment. Baseline and potential post exposure serum testing was performed on the involved employee through our Occupational Health Services department, with no noted significant change. A thorough investigation of the incident including equipment and techniques was completed and documented. We have attached the relevant reports relating to the initial response and investigation into this incident. Because of the lack of negative sequelae, there was extensive discussion as to the significance of this incident relevant to reporting as required by Section IV-B-2b-(7) of the NIH Guidelines, thus the delay in notifying the OBA.

If you have any questions regarding this matter please do not hesitate to contact Mr. Joseph Kozlovac or me at (301) 846-1451.

Respectfully,

Randall S. Morin

Randall S. Morin, Dr.P.H., RBP
Chair, NCI-Frederick Institutional Biosafety Committee
Director, Environment, Health and Safety Program, SAIC-Frederick

Attachment:

November 17, 2003 EHS Incident Report
November 17, 2003 Investigator Incident Report
November 18, 2003 NCI-Frederick IBC Minutes
November 25, 2003 BDP Investigation Report
December 16, 2003 NCI-Frederick IBC Minutes
January 20, 2004 NCI-Frederick IBC Minutes



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November 17, 2003
CA: 1579

Mr. John Eaton
Chief Government Contracting Officer
NCI-Frederick
Frederick, MD 21712

Reference: NO1-CO-12400

Dear Mr. Eaton:

SUBJECT: INCIDENT REPORT

In accordance with Article C.2.a.(7) of NCI Contract NO1-CO-12400, we are providing the attached Incident Report.

Respectfully,

SAIC-Frederick, Inc.

Randall S. Morin
Director, Environment, Health & Safety

Enclosure:

xc: L. Arthur
D. Bufter
T. Danver

EHS INCIDENT REPORT

INCIDENT TYPE: Spill Response

LOCATION: Building 434, Room 12

TIME/DATE OCCURRED: November 14, 2003

TIME/DATE REPORTED: November 14, 2003

REPORTED BY: Joseph Kozlovac
Safety Officer, EHS

SUMMARY:

See attached EHS Response Record #03-216

EHS RESPONSE RECORD

Environment, Health and Safety Program

Record #03-216

Section I - Caller Information

Name of Caller Dr. Personal Info	Extension 1012	Building and Room Number 434 Room 12
Date and Time of Call 11/14/2003 1:45pm	Date and Time of Event 11/14/2003 Approximately 1:30pm	

Section II - Type of Event

Check Type of Event <input type="checkbox"/> Medical Emergency <input type="checkbox"/> Odor <input type="checkbox"/> Exposure <input checked="" type="checkbox"/> Spill <input type="checkbox"/> Improper Waste Disposal <input type="checkbox"/> Other	Employee Injuries: <input type="checkbox"/> Yes <input type="checkbox"/> No (Have caller contact OHS at 911) Is assistance needed? <input type="checkbox"/> Yes <input type="checkbox"/> No Qty of spill: App. 200-300mL Total tube volume app. 1,000mL Name of Material: HSV-I	<input type="checkbox"/> Chemical <input type="checkbox"/> Radiation <input type="checkbox"/> Biological <input type="checkbox"/> Other
--	--	--

Section III - Detailed Event Data

Subject of Call Centrifuge tube failure resulting in spill and potential aerosol exposure.

To be completed by EHS Responder

Action Taken See attached. Time Arrived on Scene: 2:00pm

Recommendations and Follow-up Action See attached.

Call Taken By: Joseph Kozlovac

EHS Responder: Joseph Kozlovac

Date: 11/14/2003

Section IV - Completed by EHS Manager or Safety Officer

Status ☒ Pending further action

☒ Closed

BUILDING 434 SPILL RESPONSE

On November 14, 2003 at approximately 1:45 PM, Dr. [Personal Info] contacted Mr. Joseph Kozlovac to report a spill of HSV1, which involved a centrifuge tube failure. Dr. [sonal I] related to Mr. Kozlovac that he had just started the centrifuge and it was spinning up to the 15,000 RCF, which was the speed Dr. [sonal I] had set for this particular run. Approximately 2-3 minutes into the run the centrifuge made some loud noises and began to stop. Dr. [sonal I] opened the centrifuge lid as soon as he was able to and noticed liquid medium was dripping from the centrifuge and that all four tubes had failed spilling some of their content within the centrifuge. Dr. [sonal I] closed the lid at that time and evacuated the lab securing the lab as he left.

Mr. Kozlovac requested that Dr. [sonal I] deny access until he dispatched Protective Services Officers to secure the scene. Mr. Kozlovac then contacted OHS Manager Carol Tobias to inform her that there was a potential aerosol exposure to HSV1. Ms. Tobias informed Mr. Kozlovac that Dr. [sonal I] should report to OHS and take a full body shower. Mr. Kozlovac when he arrived onsite at 434 instructed Dr. [sonal I] to report to 426 and take a full shower and then report to OHS immediately after showering for evaluation. Mr. Kozlovac contacted Ms. Kathy Miller and informed her that he needed to speak to Dr. Mitra immediately. Mr. Kozlovac spoke to Dr. Mitra and briefed him on the situation, Mr. Kozlovac requested that Dr. Mitra have Dr. Steve Giardina and Dr. Jinhua Lu report to 434 ASAP.

Mr. Kozlovac in contact with the EHS office requested that a digital camera and a copy of the registration information be sent over to him. Mr. Kozlovac also requested that the EHS office contact FME so that room air exchange rates could be calculated. Dr. Giardina and Dr. Lu arrived on site and Mr. Kozlovac briefed them on the situation. Mr. Kozlovac requested that Dr. Lu obtain three, powered airpurifying respirators (PAPR) equipped with HEPA Filters. The lab was equipped with spill response equipment however the disinfectant that they had on hand in the lab was not the best choice for a largescale wipe down of work surfaces. Mr. Kozlovac contacted EHS and requested that a case of Cavicide (quaternary ammonium compound) be delivered to 434 loading dock. Dr. Lu returned with the PAPRs. Dr. [sonal I] returned from OHS shortly there after.

Dr. Morin contacted Mr. Kozlovac and based on data obtained from FME that the room air exchange rate was approximately 17 ACH. Approximately 50-60 minutes had passed since Dr. [sonal I] had left the laboratory. Mr. Kozlovac based upon the available data decided to make entry into the laboratory wearing tyvek suit, booties, double gloves and PAPRs. Dr. [sonal I] and Dr. Lu accompanied Mr. Kozlovac.

Mr. Kozlovac assessed the situation and took a number of digital photographs. The tubes still contained a majority of the culture liquid. This material was placed in a secondary container and transported to the biological safety cabinet. The culture material was disinfected using a 1:10 final volume solution of 5.25% Sodium hypochlorite and media/tap water. The conical tubes were also soaked in the solution. Mr. Kozlovac requested that Dr. [sonal I] and Dr. Lu focus on decontaminating laboratory surfaces with

Cavicide. Mr. Kozlovac poured Cavicide into the centrifuge bowl and sprayed the sides for disinfection purposes. Using forceps Mr. Kozlovac removed plastic shards from the interior of the centrifuge bowl, placing recovered shards into a nalgene pipette tray that contained Cavicide. Remaining disfectant was absorbed with laboratory towels and discarded into autoclave bags. A final decontamination of the centrifuge was conducted again using Cavicide. Dr. [sonal I] left the laboratory when the PAPR he was wearing was running out of charge. Mr. Kozlovac requested that Dr. [sonal I] obtain a mop and bucket in order to decon the floor. Mr. Kozlovac and Dr. Lu finished decontaminating worksurfaces and walls.

Mr. Kozlovac and Dr. Lu exited the lab at approximately 3:40 PM. Dr. [sonal I] indicated to Mr. Kozlovac that he and Dr. Lu would handle the disinfection of the laboratory floor. Dr. [sonal I] returned to OHS for further consultation at OHS request.

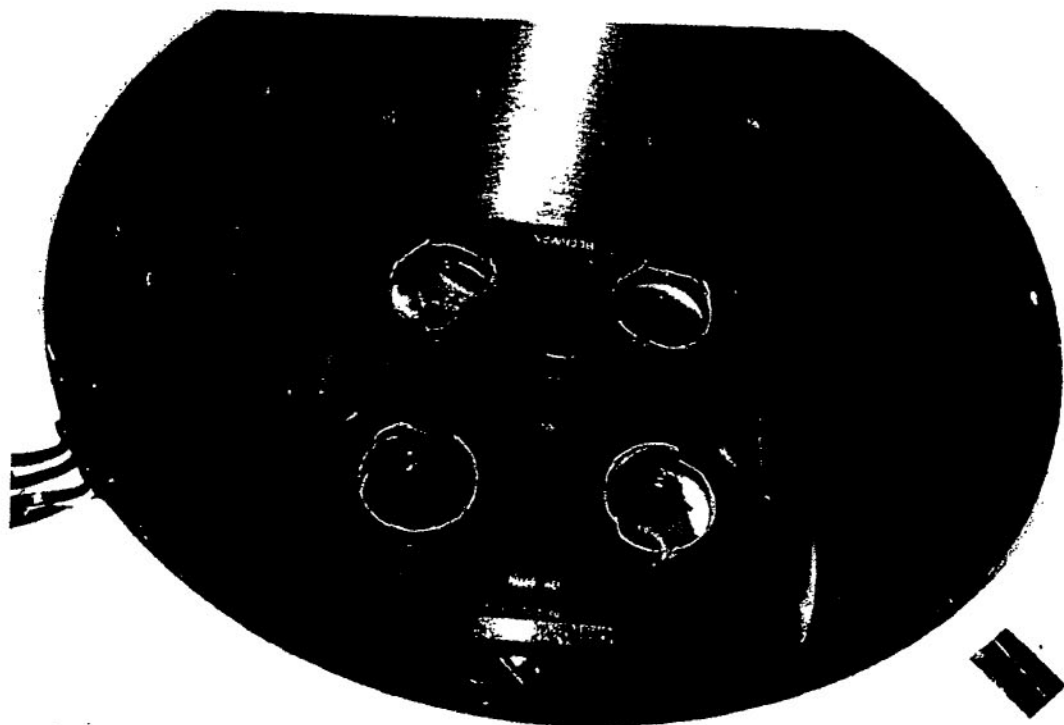
OHS Medical Response:

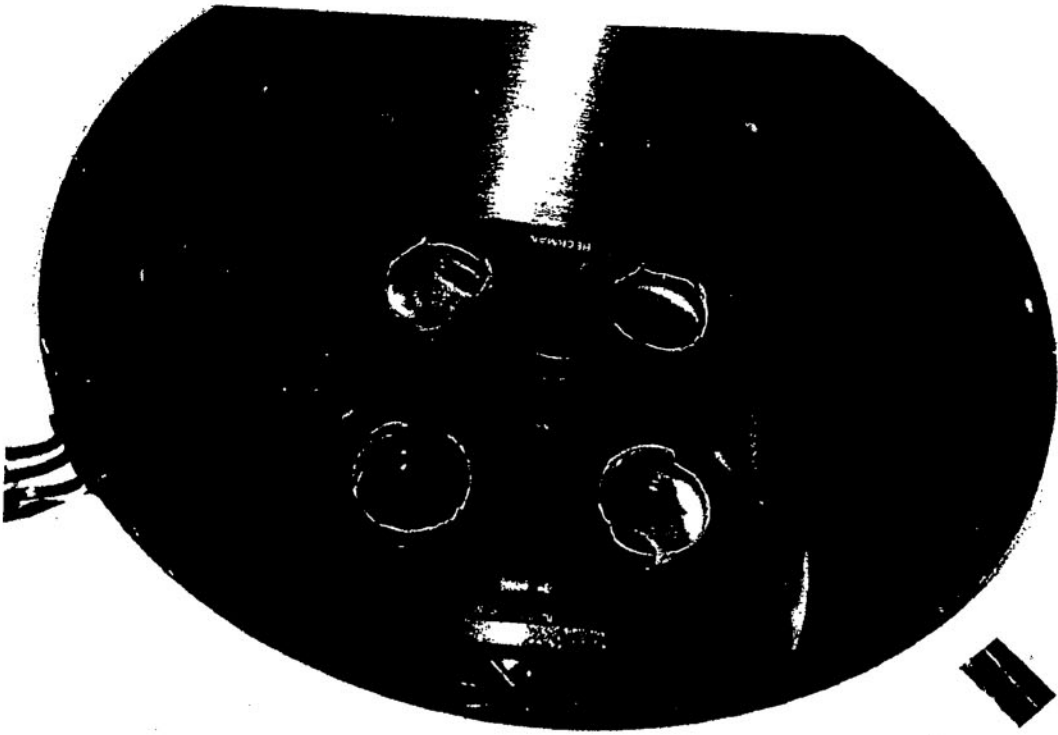
At the time the protocol was submitted to the IBC, a unique medical surveillance program was developed for this project. Working in concert with the CDC, individual serum responses were obtained on all appropriate staff.

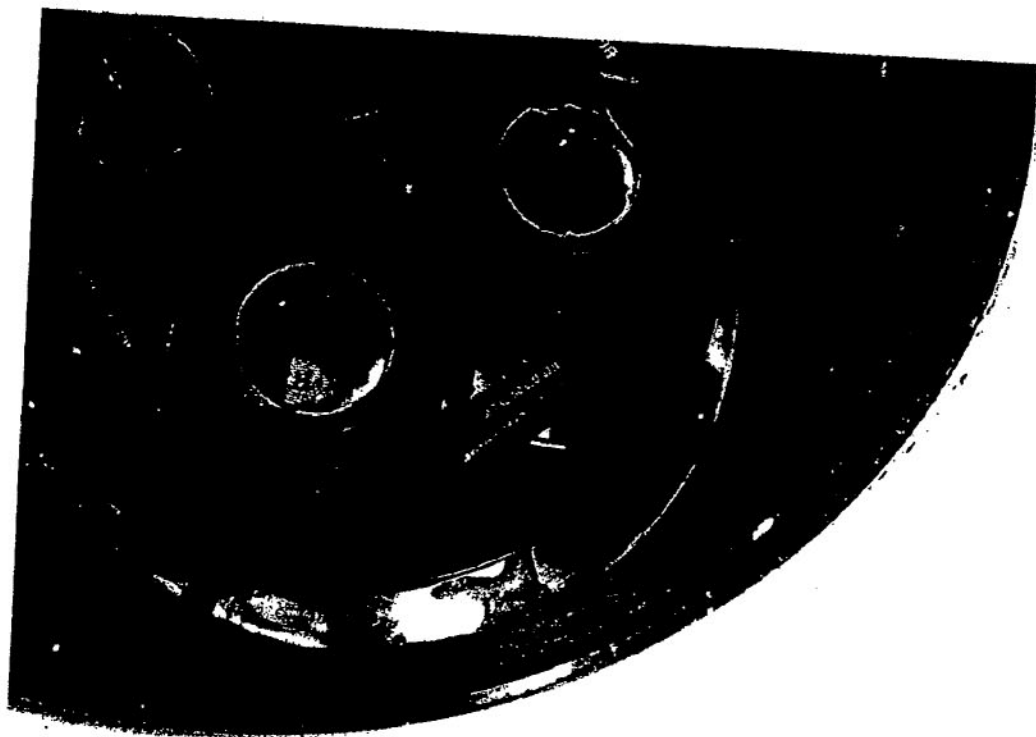
Following the potential exposure incident, the employee's laboratory results were pulled and decisions about potential post-exposure were made immediately per protocol. Determinations were checked with the CDC for concurrence, which was obtained.

EHS Recommendations:

1. BDP should take the lead role in conducting an investigation into the root cause of the centrifuge tube failure with assistance and input from EHS as necessary. BDP should provide a copy of this investigation to EHS for comment and for file.
2. Dr. [sonal I] should provide OHS titer information on the spilled material when available.
3. BDP and EHS staff that participated in the spill response should hold a joint debriefing and lessons learned session.







Incident Report
November 17, 2003

Personal Info

Ph.D. and Jinhua Lu, Ph.D.

When: November 14, 2003

Where: Bldg 434/ rm 12

Hazard: Recombinant herpes simplex virus type 1 infected cells and supernatant, infectious and replication competent, approximately 900ml with about 250ml spilled.

Personal protection equipments used: Lab coat, goggles, and boot covers

Report: Harvested infected cells and supernatant containing recombinant herpes simplex virus type 1 was being centrifuged (Beckman Model J-25I, NCI #C119983, rotor #JS-7.5) in bldg 434 room 12 at approximately 2pm on November 14, 2003. Infectious materials were held in 4 Corning 250ml conical centrifuge tubes (cat #430776, lot #16701010, BDP #20141). Centrifuge was set for 1,500 x g RCF for 5 minutes at 16°C. Centrifugation was started and I turned away to set up other items. Within 30 seconds into the centrifugation, a loud noise was heard. The centrifuge decelerated but did not come to a complete stop, so I manually stopped it by depressing the stop button. I open the centrifuge door and saw that all four tops of the tubes were sheared off with infectious media covering the chamber and door of the centrifuge. Infectious media dripped from the inside of the centrifuge door. My immediate response was to disinfect the door with Dispatch. Then, I realized this was not spill procedure so I discarded the wipes in an autoclave container and shut the centrifuge door. I inspected the lab coat and goggles for any sign of splash but did not see any. I left the room and proceeded to call Dr. Jinhua Lu (supervisor), Dr. Steve Giardina (BDP safety officer), and Mr. Joseph Kozlovac (Biosafety officer).

The first response was Security; they proceeded to tie off access to room 12. Mr. Kozlovac arrived shortly and I then reported to OHS. When I returned, Dr. Lu and Dr. Giardina were already there. Mr. Kozlovac, Dr. Lu, and myself donned PAPR protective gear and entered room 12. Initial inspection was followed by disinfection using Cavicide. Mr. Kozlovac and Dr. Lu disinfected the centrifuge and its contents with a combination of Cavicide and 5-10% bleach. I disinfected all surrounding surfaces with Cavicide and mopped the floor with 5-10% bleach.

Possible causes for this accident: Dr. Lu and I inspected the tubes and centrifuge rotor and found that the tubes were too tall for this rotor even though it fits perfectly in the buckets. We suspect that the tops were sheared off as a result of the buckets swinging out the tops coming in contact with the rotor.

Preventive measures: (1). The laboratory personnel shall inspect the centrifugation bottles/tubes and test to see if they are fit for centrifugation in the rotor before running the actual samples. (2). The safety policy and procedures will be reviewed and discussed to

all people in the laboratory. If necessary, people will be trained on pertinent regulations and applicable laboratory procedures.



**NCI-FREDERICK
INSTITUTIONAL BIOSAFETY COMMITTEE**

**Minutes – Meeting November 18, 2003
NCI-Frederick**

The NCI-Frederick Institutional Biosafety Committee was convened at 12:15 p.m. in the Building 549 Executive Boardroom with the following members in attendance:

Dr. Randall Morin, Chair
Mr. Joseph Kozlovac, Secretary
Dr. Bruce Crise
Dr. Steve Hughes

Dr. David Garfinkel
Dr. Henry Hearn
Dr. Michael Baseler

Members not in attendance: Dr. Stephen Creekmore, Dr. Donald Court, Dr. Jeanne Herring, Dr. Melinda Hollingshead, Mr. Lucien Winegar, Ms. Carol Ingraham Tobias, Ms. Cheryl Parrott (Ex Officio)
Others in attendance: Ms. Cara Lamberson, Mr. Tom Denver

INTRODUCTION

Dr. Morin called the meeting to order.

REVIEW OF PROTOCOLS

Dr. Crise introduced a recombinant DNA registration entitled, *Generation of DNA vectors for functional analysis of Tumor Endothelial Markers (TEMs)* submitted by Dr. Brad St. Croix. Dr. Crise reviewed the particulars of the proposal and wanted to know if the animal study was approved yet. Mr. Kozlovac informed him that this was a simultaneous submission and the IACUC was currently reviewing the animal study. No other issues were brought up concerning this registration.

Conditionally Approved

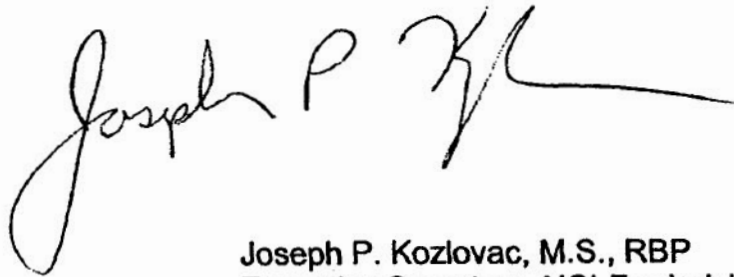
1. Provide animal personnel signatures
2. Animal facility manager signature
3. Receive ACUC approval for the Animal Study Proposal

OTHER BUSINESS

Mr. Kozlovac informed the committee about the centrifuge accident on November 14, 2003. He also read to the committee an email from Ms. Tobias concerning the accident. Dr. Hughes recommended sending out a safety reminder of the steps to take after a centrifuge accident. He also recommended that the IBC request a report from the BDP on the incident and their findings. All concurred and Dr. Morin agreed to draft a memo to Dr. Mitra requesting the report.

Meeting Adjourned: 1:03 pm

Respectfully submitted,



Joseph P. Kozlovac, M.S., RBP
Executive Secretary, NCI-Frederick
Institutional Biosafety Committee

Approved: 

Randall S. Morin, Dr. P.H.
Chairman, NCI-Frederick IBC

xc: Each Committee Member
Dr. Wiltrout
Dr. Reynolds
Mr. Eaton
Dr. Arthur
Mr. Bufter



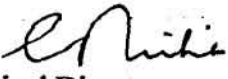
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MEMORANDUM

Date: November 25, 2003



To: Mr. Joseph Kozlovac
Biological Safety Officer, EHS

Thru: Dr. George Mitra 
Program and Technical Director
Biopharmaceutical Development Program (BDP)

From: Dr. Steven L. Giardina 
Safety Officer
Biopharmaceutical Development Program (BDP)

Subject: **INVESTIGATION REGARDING THE ROOT CAUSE OF THE
RECOMBINANT HERPES VIRUS SPILL OF 11/14/03**

The immediate cause of the spill as described in Attachment 1 (Memorandum from Dr. Giardina to Mr. Kozlovac, dated November 21, 2003, entitled "INVESTIGATION OF VIRUS SPILL IN BLDG. 434, RM. 12") was the use of the 250ml Corning Centrifuge Tubes in a Beckman JS-7.5 rotor. The JS-7.5 rotor is an open, swinging bucket type rotor. The Corning tubes could not clear the Beckman Model J-26i spindle as the rotor accelerated to the set speed. As a result, the tops of the centrifuge tubes came into direct contact with the spindle leading to loss of container integrity and spilling of the contents. Additionally, a U-shaped insert was used in each carrier rather than the appropriate V-shaped insert recommended for use with conical centrifuge tubes.

The underlying root cause is inadequate training on the safe operation of a centrifuge in combination with a lack of appreciation of the potential consequences of operating the centrifuge incorrectly. This lack of training includes being unaware of an existing Standard Operating Procedure governing the handling of Herpes Virus in Room 12 of Building 434. Specifically, SOP 22923 (Attachment 2) clearly states that rotor buckets must be equipped with covers. However, as a result of the 11/14/03 incident, a revision has been made to the SOP to ensure that this is more clearly defined (Attachment 3). When interviewed by the BDP Safety Officer, Dr.  indicated that he was unaware of this SOP and was not trained on it. Since Dr. R. Harris transmitted an e-mail (Attachment 4) prior to the incident that specifically addresses the need for completion of a Safety-related curriculum, it is not clear why Dr.  was unaware of this SOP. Taken together, these findings indicate emphasizing the importance of safety training with renewed vigor to all members of the BDP.

Several remedies have been initiated by BDP Management to address the need for remedial training and increasing awareness of the consequences of ignoring proper safety procedures.

These include:

1. Establishing a BDP Safety Committee composed of both supervisory and staff level employees. The EHS Biological Safety Officer will be a member of this committee. The first meeting has already been set for early January 2004. This committee will meet on a quarterly basis at a minimum to discuss training and risk assessment issues for new and ongoing projects.
2. In conjunction with BDP Quality Assurance, an enhanced safety curriculum will be established. This curriculum will define the type of training required of BDP staff. This training will be renewed annually in conformance with BDP's basic requirements for operator training.
3. In collaboration with EHS, mandatory training sessions will be set up as soon as possible for all employees who operate centrifuges, autoclaves or other equipment where the potential for serious injury exists. An SOP that specifically addresses handling an infectious material spill in a centrifuge will be drafted and implemented.
4. The BDP Safety Officer will write and distribute, through BDP Senior Management, a memorandum to all BDP employees reemphasizing the expectations regarding safety training.

It is our belief that establishing appropriate safety curricula in combination with a Program-wide forum to anticipate and address safety-related issues will minimize the opportunity for a repetition of this or other potentially injurious incidents.

SG/km

Xc: Dr. Creekmore
Mr. Gaum
Mr. Rothchild
Dr. Michiel
Dr. Lu
Dr. sonal I
Dr. Harris

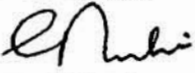



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MEMORANDUM

Date: November 21, 2003

To: Mr. Joseph Kozlovac
Biological Safety Officer, EHS

Thru: Dr. George Mitra 
Program and Technical Director
Biopharmaceutical Development Program (BDP)

From: Dr. Steven L. Giardina 
Safety Officer
Biopharmaceutical Development Program (BDP)

Subject: INVESTIGATION OF VIRUS SPILL IN BLDG. 434, RM. 12

On Friday, November 14, 2003, I was called out of Dr. Creekmore's weekly meeting by Dr. Mitra and informed that there had been a virus spill in Building 434, Room 12. Dr. Personal Info Development Scientist, Virology Laboratory, BDP, had been exposed to culture media containing a recombinant Herpes virus (see Protocol #P150703RHA01). Dr. Jinhua Lu (Manager, Virology Laboratory) and I left the meeting and arrived at Lab 12 to find the area cordoned off by Security and Mr. Kozlovac. I was informed that Dr. sonal I had already departed to OHS for a shower and, potentially, to have his blood drawn.

Mr. Kozlovac was in contact with Dr. Morin to determine the number of air changes per hour so that an appropriate time could be allotted for clearing the room air of aerosolized virus. While we were waiting for this information, Dr. sonal I returned in scrubs. At Mr. Kozlovac's request he recounted the sequence of events.

Dr. sonal I was working in Lab 12 to collect approximately 800ml of Herpes virus-containing supernatant. He aliquoted the material into four conical Falcon centrifuge tubes and "eyeballed" the fluid levels since a balance was not available. He placed the tubes into the a swinging bucket, open-rotor, set the speed to 1500 (RPM?), turned the unit on and then turned away from the centrifuge to perform another task. He heard "noise" and noted that the centrifuge had probably not reached the set speed before the noise started. Once the centrifuge had stopped, he opened the lid and noted liquid on the interior of the centrifuge's lid. All four tubes had broken. He began to clean the lid and then realized that this was probably not the best idea. He closed the centrifuge, removed his contaminated gloves, lab coat and goggles, and called BDP personnel, including myself. He called EHS who then responded promptly to the spill.

Dr. Morin subsequently informed us that the room had approximately 17 air changes per hour. Mr. Kozlovac felt that sufficient time had elapsed for safe entry into the room. PAPRs were obtained from the BL-3 suite of Building 459 and he and Drs. [redacted] and [redacted] entered the room to initiate spill clean up. Upon opening the centrifuge, the buckets were placed into a tray containing diluted bleach, the fragments of centrifuge tubes removed with forceps and the interior of the centrifuge cleaned with Cavicide. Dr. [redacted] cleaned the walls, counter tops and other accessible surfaces. Dr. [redacted] had to depart early when his respirator unit's battery pack failed. By this time, however, most of the cleaning had been completed. Mr. Kozlovac left the room under continuing quarantine pending cleaning the floor. The total operation was completed in approximately 2.5 hours.

Should you require any additional information, you may reach me at ext. 1821.

SG/km

xc: Dr. Creekmore
Mr. Gaum
Mr. Rothchild
Dr. Michiel
Dr. Lu
Dr. [redacted]
Dr. Harris

National Cancer Institute-Frederick
Frederick, MD

Biopharmaceutical Development Program

STANDARD
OPERATING
PROCEDURE

Effective Date

SEP 30 2003

Procedure Number

22923

Page 1 of 7

Revision 00

Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

Author/Date: Judith Pooley-Nelson 9/11/03 Judith Pooley-NelsonApprovals/Date: 12114
Director 12114 12114 12114
BQC 12114 12114 12114
BQA 12114 12114 12114SOP References: 12114
12114 12114 12114

Supersedes: NA

Purpose: This procedure outlines the process for handling viruses by BQC in BL2 laboratories.Scope: This procedure applies to BDP QC personnel specifically trained in handling BSL-2 level or greater pathogenic agents, directed by competent scientists. Other personnel within the BDP may use this SOPContents:

- 1.0 Authority and Responsibility
- 2.0 Laboratory Facilities
- 3.0 Materials
- 4.0 Safety Equipment (Primary Barriers)
- 5.0 Viral Spills
- 6.0 Cleaning/Sanitization of BSC
- 7.0 Handling Uninfected and Virus Infected Cultures
- 8.0 Decontamination of Waste
- 9.0 Documentation
- 10.0 References

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STANDARD OPERATING PROCEDURE

Effective Date

SEP 30 2003

Page 2 of 7

Procedure Number

22923

Revision 00

Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

1.0 Authority and Responsibility

- 1.1 The Director, Biopharmaceutical Quality Control (BQC) has the authority to define this procedure.
- 1.2 BQC personnel are responsible for the accurate performance of this procedure.
- 1.3 BQC personnel are responsible for maintaining records pertaining to this procedure.
- 1.4 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

2.0 Laboratory Facilities

NOTE: When viruses are being handled, the laboratory is posted with biohazard signs restricting access to the laboratory.

- 2.1 The laboratories are provided with lockable doors.
- 2.2 Each laboratory contains a sink. Untreated virus solutions cannot be discarded down the sink. All solutions that have come into contact with virus must first be inactivated with bleach, then the solutions can be discarded down the sink with water running.
- 2.3 The laboratories are designed to be cleaned easily.
- 2.4 Bench tops are impervious to water and resistant to moderate heat and organic solvents, acids, alkalis and chemicals used to decontaminate work surfaces.
- 2.5 Laboratory furniture is covered with non fabric material for easy decontamination.
- 2.6 Biological safety cabinets are located such that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets (BSC) to operate outside their containment parameters.
- 2.7 An eyewash station is readily available.
- 2.8 Illumination is adequate for the activities performed in the room.
- 2.9 All pipetting is performed with manual or automatic pipetting devices (No mouth pipetting).



STANDARD OPERATING PROCEDURE

Effective Date

SEP 30 2003

Procedure Number

22923

Page 3 of 7

Revision 00

Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

2.10 A shower is located in Bldg 433 outside of room 12.

3.0 Materials

3.1 Approved Cleaning/Sanitization Agents

3.1.1 Dispatch (BDP PN 10167) or equivalent.

3.1.2 Septihol (BDP PN 30129) or equivalent. Use as a sanitizing agent.

3.1.3 Clorox bleach (BDP PN 20295) or equivalent. Clorox with this BDP PN is supplied as 6.15% bleach.

3.1.4 Spor-Klenz (BDP PN 10163) or equivalent.

3.1.5 Cavicide (BDP PN 10168) or equivalent.

NOTE: Rotate Dispatch, Spor-Klenz, Cavicide and undiluted Clorox bleach for decontamination every other cleaning. Aliquotted bleach should be <24 hours old. Do not use Cavicide with Dispatch, Clorox or Spor-Klenz.

3.2 24 x 36 autoclave bag or equivalent, BDP P/N 20728.

3.3 30 x 36 polypropylene autoclave bag or equivalent, BDP P/N 20665.

3.4 Vacuum flasks for aspiration of medium and samples.

3.5 Aspirating pipettes 2 mL (BDP PN 21331) or 5 mL (BDP PN 21330).

3.6 Terminal pipette keepers (BDP PN 21338).

3.7 Dedicated waste cart labeled with building and room number, as appropriate.

3.8 Povidone-iodine lotion (Supplied by OHS)

3.9 Autoclave Indicator Strips BDP PN 20316 or equivalent.

3.10 Handwash: Softcide BDP PN 30137 or equivalent.

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STANDARD OPERATING PROCEDURE

Effective Date

SEP 30 2003

Procedure Number

22923

Page 4 of 7

Revision 00

Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

4.0 Safety Equipment (Primary Barriers)

- 4.1 Work is performed in Class II BSC's or with physical containment (i.e., glove bags) in other areas of the laboratory.
- 4.2 Face protection (safety glasses or face shield) are used in the laboratory.
- 4.3 Protective laboratory coats or uniforms designated for laboratory use are worn in the laboratory. This protective clothing is removed and left in the laboratory.
- 4.4 Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. At times it may be appropriate to wear two pairs of gloves and additional protective sleeves depending on the type of manipulation (when working with a liter or $\geq 1 \times 10^8$ infectious unit/mL. Hands are washed with Softcide or equivalent following removal of gloves.
- 4.5 Fluids are removed from cultures using sterile individually wrapped plastic aspirating pipettes and fluids are contained in the aspirating flask to which the decontamination agent has been added. The aspirating flask containing culture material is in series with another flask containing decontamination agent (as an emergency overflow) and is in series with a 0.2 μ fluid retention filter before connection to the vacuum system.
- 4.6 All pipettes are rinsed in undiluted Clorox bleach before placing in the terminal pipette keeper prior to disposal or autoclaving.

5.0 Viral Spills

NOTE: A spill within a functioning BSC or other containment device does not ordinarily constitute a reportable spill. Since the quantity of infectious material will not exceed one liter, which could be contained within the work area of the BSC, no reportable spills will be anticipated within the BSC. Any spill occurring outside the BSC or other containment device is a reportable spill. For reportable spills contact the Supervisor as soon as the situation is stabilized.

- 5.1 If virus containing fluids or virus-infected cells are spilled the material must immediately be contained and disinfected. This is best accomplished by putting a towel soaked in the decontamination fluid over the spill. Additional decontamination fluid may then be gently poured onto the towel



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and allowed to remain in contact for at least 30 minutes (this avoids aerosol which may arise from spraying the spilled material directly.

- 5.2 If personnel were exposed, immediately remove laboratory clothing and wash exposed skin with Povidone-iodine lotion followed by soap and water.
- 5.3 If an eye exposure is suspected, flush eyes with copious volumes of water at an eyewash station for 15 minutes.
- 5.4 **Exposed personnel must report immediately after emergency action to Occupational Health Services for a post-exposure evaluation. The supervisor must complete an Accident Report Form.**

6.0 Cleaning/Sanitization of BSC

- 6.1 Wipe down work surfaces with the sanitization agent before beginning work.
- 6.2 Clean the laboratory equipment (e.g., centrifuges, incubators, waterbaths, etc.) according to **SOP 22909 - Cleaning and Disinfection of Equipment and Bench Tops in BQC.**
- 6.3 After working in the BSC decontaminate with Dispatch, Cavicide or Spc-Klenz, based on the rotational schedule.

7.0 Centrifugation of virus containing material.

- 7.1 Low speed centrifugation. The rotor buckets must be equipped with covers. If removable, the bucket and cover should be brought to the BSC, tubes placed into the bucket, covers affixed before placing them back in the centrifuge. If rotor buckets are affixed to the rotor the samples may be brought to the centrifuge placed into the bucket and covers affixed.
- 7.2 Ultracentrifugation. The rotor should be brought to the hood and the samples placed into the rotor, the cover affixed and the rotor placed into the centrifuge.
- 7.3 The rotors, buckets, covers and centrifuge should be disinfected following each days use according as in 6.0.

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8.0 Handling uninfected and virus infected cultures.

- 8.1 Maintain separate bottles of medium for each uninfected and virus infected cell culture.
- 8.2 Always handle the uninfected cultures first and sanitize the BSC.
- 8.3 Viral cultures are handled after all uninfected cultures have been manipulated.
- 8.4 The BSC is decontaminated as described in 6.0.

9.0 Decontamination of Waste

- 9.1 Decontaminate liquids and perishables with the addition of sufficient undiluted Clorox bleach, to account for at least 10% of the final volume of liquids and mix. Allow to remain in contact for at least 30 minutes.
- 9.2 Dispose of decontaminated liquids down the drain while running tap water.
- 9.3 Place solids, including used gowning materials, in 24 x 36 inch or 30 x 36 inch autoclave bags and seal appropriately. Double-bag into a 30 x 36 inch polypropylene bag and seal appropriately. Include indicators in the inside bag to validate the autoclave run at least once during the week in which trash is autoclaved. Place the double-bagged waste into the designated trash container for transport to the autoclave room in Building 472 (adjacent to Building 433). Autoclave waste according to **SOP 12114. (Operation of the 65300 Autoclave, Building 472)**. Following autoclaving the indicators may be incubated for at least 48 hours in the BQC incubator set at 55°C-60°C. Follow instructions with the indicators for preparing them for incubation following autoclaving. Include one unautoclaved indicator with each set as a positive control. Following autoclaving, waste is placed into a red bag the bag sealed and placed in the grey biohazard waste cart directly outside the building.

10.0 Documentation

- 10.1 Record disposal and removal in the Laboratory Notebook.
- 10.2 Record hood usage and cleaning on form 22909-01.



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11.0 References

- 11.1 Biological Safety in Microbiological and Biomedical Laboratories, Center for Disease Control and Prevention and National Institutes of Health. Current version.
- 11.2 NCI-Frederick Bloodborne Pathogen Exposure Control Plan, current version
- 11.3 NCI-Frederick Environmental Health, and Safety Program. Health, Safety and Environmental Compliance Program Manual. Current version.
<http://web.ncifcrf.gov/campus/safety/compliance/index.stm>
- 10.4 Environmental, Health and Safety. Safetygrams. Current version.

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National Cancer Institute-Frederic.
Frederick, MD



Biopharmaceutical Development Program

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Revision 01

Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

Author/Date: _____ Judith Poiley-Nelson

Approvals/Date: _____
 Director
 BQC
 BQA

SOP References: 01304, 22909

Supersedes: Revision 00

Purpose: This procedure outlines the process for handling viruses by BQC in BL2 laboratories.

Scope: This procedure applies to BDP QC personnel specifically trained in handling BSL-2 level or greater pathogenic agents, directed by competent scientists. Other personnel within the BDP may use this SOP

Contents:

- 1.0 Authority and Responsibility
- 2.0 Laboratory Facilities
- 3.0 Materials
- 4.0 Safety Equipment (Primary Barriers)
- 5.0 Viral Spills
- 6.0 Cleaning/Sanitization of BSC
- 7.0 Handling Uninfected and Virus Infected Cultures
- 8.0 Decontamination of Waste
- 9.0 Documentation
- 10.0 References

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Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

1.0 Authority and Responsibility

- 1.1 The Director, Biopharmaceutical Quality Control (BQC) has the authority to define this procedure.
- 1.2 BQC personnel are responsible for the accurate performance of this procedure.
- 1.3 BQC personnel are responsible for maintaining records pertaining to this procedure.
- 1.4 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

2.0 Laboratory Facilities

NOTE: When viruses are being handled the laboratory is posted with biohazard signs restricting access to the laboratory.

- 2.1 The laboratories are provided with lockable doors.
- 2.2 Each laboratory contains a sink. Untreated virus solutions cannot be discarded down the sink. All solutions that have come into contact with virus must first be inactivated with bleach, then the solutions can be discarded down the sink with water running.
- 2.3 The laboratories are designed to be cleaned easily.
- 2.4 Bench tops are impervious to water and resistant to moderate heat and organic solvents, acids, alkalis and chemicals used to decontaminate work surfaces.
- 2.5 Laboratory furniture is covered with non fabric material for easy decontamination.
- 2.6 Biological safety cabinets are located such that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets (BSC) to operate outside their containment parameters.
- 2.7 An eyewash station is readily available.
- 2.8 Illumination is adequate for the activities performed in the room.
- 2.9 All pipetting is performed with manual or automatic pipetting devices (No mouth pipetting).



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2.10 A shower is located in Bldg 433 outside of room 12.

3.0 Materials

3.1 Approved Cleaning/Sanitization Agents

3.1.1 Dispatch (BDP PN 10167) or equivalent.

3.1.2 Septihol (BDP PN 30129) or equivalent. Use as a sanitizing agent.

3.1.3 Clorox bleach (BDP PN 20295) or equivalent. Clorox with this BDP PN is supplied as 6.15% bleach.

3.1.4 Spor-Klenz (BDP PN 10163) or equivalent.

3.1.5 Cavicide (BDP PN 10168) or equivalent.

NOTE: Rotate Dispatch, Spor-Klenz, Cavicide and undiluted Clorox bleach for decontamination every other cleaning. Aliquotted bleach should be <24 hours old. Do not use Cavicide with Dispatch, Clorox or Spor-Klenz.

3.2 24 x 36 autoclave bag or equivalent, BDP P/N 20728.

3.3 30 x 36 polypropylene autoclave bag or equivalent, BDP P/N 20665.

3.4 Vacuum flasks for aspiration of medium and samples.

3.5 Aspirating pipettes 2 mL (BDP PN 21331) or 5 mL (BDP PN 21330).

3.6 Terminal pipette keepers (BDP PN 21338).

3.7 Dedicated waste cart labeled with building and room number, as appropriate.

3.8 Povidone-iodine lotion (Supplied by OHS)

3.9 Autoclave Indicator Strips BDP PN 20316 or equivalent.

3.10 Handwash: Softcide BDP PN 30137 or equivalent.



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4.0 Safety Equipment (Primary Barriers)

- 4.1 Work is performed in Class II BSC's or with physical containment (i.e., glove bags) in other areas of the laboratory.
- 4.2 Face protection (safety glasses or face shield) are used in the laboratory.
- 4.3 Protective laboratory coats or uniforms designated for laboratory use are worn in the laboratory. This protective clothing is removed and left in the laboratory.
- 4.4 Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. At times it may be appropriate to wear two pairs of gloves and additional protective sleeves depending on the type of manipulation (when working with a liter or $\geq 1 \times 10^8$ infectious unit/mL. Hands are washed with Softcide or equivalent following removal of gloves.
- 4.5 Fluids are removed from cultures using sterile individually wrapped plastic aspirating pipettes and fluids are contained in the aspirating flask to which the decontamination agent has been added. The aspirating flask containing culture material is in series with another flask containing decontamination agent (as an emergency overflow) and is in series with a 0.2μ fluid retention filter before connection to the vacuum system.
- 4.6 All pipettes are rinsed in undiluted Clorox bleach before placing in the terminal pipette keeper prior to disposal or autoclaving.

5.0 Viral Spills

NOTE: A spill within a functioning BSC or other containment device does not ordinarily constitute a reportable spill. Since the quantity of infectious material will not exceed one liter, which could be contained within the work area of the BSC, no reportable spills will be anticipated within the BSC. Any spill occurring outside the BSC or other containment device is a reportable spill. For reportable spills contact the Supervisor as soon as the situation is stabilized.

- 5.1 If virus containing fluids or virus-infected cells are spilled the material must immediately be contained and disinfected. This is best accomplished by putting a towel soaked in the decontamination fluid over the spill. Additional decontamination fluid may then be gently poured onto the towel.



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and allowed to remain in contact for at least 30 minutes (this avoids aerosol which may arise from spraying the spilled material directly.

5.2 If personnel were exposed, immediately remove laboratory clothing and wash exposed skin with Povidone-iodine lotion followed by soap and water.

5.3 If an eye exposure is suspected, flush eyes with copious volumes of water at an eyewash station for 15 minutes.

5.4 **Exposed personnel must report immediately after emergency action to Occupational Health Services for a post-exposure evaluation. The supervisor must complete an Accident Report Form.**

6.0 Cleaning/Sanitization of BSC

6.1 Wipe down work surfaces with the sanitization agent before beginning work.

6.2 Clean the laboratory equipment (e.g., centrifuges, incubators, waterbaths, etc.) according to *SOP 22909 - Cleaning and Disinfection of Equipment and Bench Tops in BQC*.

6.3 After working in the BSC decontaminate with Dispatch, Cavicide or Spor-Klenz, based on the rotational schedule.

7.0 Centrifugation of virus containing material.

7.1 Low speed centrifugation. The rotor buckets must be equipped with covers. If removable, the bucket and cover should be brought to the BSC, tubes placed into the bucket, covers affixed before placing them back in the centrifuge. If rotor buckets are affixed to the rotor the samples may be brought to the centrifuge placed into the bucket and covers affixed. Centrifuge tubes/bottles must be suitable for the specific rotor used. The operator is responsible for ensuring that the container selected meets individual manufacturer's specifications.

7.2 Ultracentrifugation. The rotor should be brought to the hood and the samples placed into the rotor, the cover affixed and the rotor placed into the centrifuge.



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7.3 The rotors, buckets, covers and centrifuge should be disinfected following each days use according as in 6.0.

8.0 Handling uninfected and virus infected cultures.

8.1 Maintain separate bottles of medium for each uninfected and virus infected cell culture.

8.2 Always handle the uninfected cultures first and sanitize the BSC.

8.3 Viral cultures are handled after all uninfected cultures have been manipulated.

8.4 The BSC is decontaminated as described in 6.0.

9.0 Decontamination of Waste

9.1 Decontaminate liquids and perishables with the addition of sufficient undiluted Clorox bleach, to account for at least 10% of the final volume of liquids and mix. Allow to remain in contact for at least 30 minutes.

9.2 Dispose of decontaminated liquids down the drain while running tap water.

9.3 Place solids, including used gowning materials, in 24 x 36 inch or 30 x 36 inch autoclave bags and seal appropriately. Double-bag into a 30 x 36 inch polypropylene bag and seal appropriately. Include indicators in the inside bag to validate the autoclave run at least once during the week in which trash is autoclaved. Place the double-bagged waste into the designated trash container for transport to the autoclave room in Building 472 (adjacent to Building 433). Autoclave waste according to **SOP 12114 - Operation of the 65300 Autoclave, Building 472**. Following autoclaving the indicators may be incubated for at least 48 hours in the BQC incubator set at 55°C-60°C. Follow instructions with the indicators for preparing them for incubation following autoclaving. Include one unautoclaved indicator with each set as a positive control. Following autoclaving, waste is placed into a red bag the bag sealed and placed in the grey biohazard waste cart directly outside the building.



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Title: Procedures for Safe Handling and Decontamination of Viruses by BDP/BQC and Related Personnel

10.0 Documentation

10.1 Record disposal and removal in the Laboratory Notebook.

10.2 Record hood usage and cleaning on form 22909-01.

11.0 References

11.1 Biological Safety in Microbiological and Biomedical Laboratories, Center for Disease Control and Prevention and National Institutes of Health. Current version.

11.2 NCI-Frederick Bloodborne Pathogen Exposure Control Plan, current version

11.3 NCI-Frederick Environmental Health, and Safety Program. Health, Safety and Environmental Compliance Program Manual. Current version.
<http://web.ncifcrf.gov/campus/safety/compliance/index.stm>

11.4 Environmental, Health and Safety. Safetygrams. Current version.

Miller, Kathy

From: Harris, Raymond
Sent: Friday, November 21, 2003 4:20 PM
To: Miller, Kathy
Subject: FW: New Curriculum for handling viruses - You are a member of this new curriculum

-----Original Message-----

From: Gibson, Sandy
Sent: Monday, October 06, 2003 9:02 AM
To: Michiel, Dennis; Poiley, Judy; Bowers, Gary; Seltzer, Tina; Mowen, Cheryl; Gilbert, Harold; Staffone, Shoshanna; Soman, Gopalan; Hartmann, Wanda; Kallarakal, Abraham; Yang, Xiaoyi; Lu, Jinhua; Poon, Dexter; Artip, Moria; Zheng, Gemin; Yang, Zhiwen; Harris, Raymond; Rhodes, Mary; Giardina, Steve; Nelson, Roy (Earl); Ouellette, Tom
Subject: New Curriculum for handling viruses - You are a member of this new curriculum

All,

We have established a new training curriculum called "Virus Safety" and you have been assigned as a member of this curriculum. Currently, there is one SOP that is part of this curriculum, SOP 22923 "Procedures for Safe Handling and Decontamination of Viruses by BDP / BQC and Related Personnel". Please read this SOP and document your training on form 21600-01. (Dennis, Judy, since you are reviewers of this SOP, I already have your training documented). Questions about the content of this SOP can be directed to Judy Poiley-Nelson (author).

PLM will also alert you to this requirement.

If you have questions, please call me.

Sandy Gibson (x6927)
QA Training Manager



**NCI-FREDERICK
INSTITUTIONAL BIOSAFETY COMMITTEE**

**Minutes – Meeting December 16, 2003
NCI-Frederick**

The NCI-Frederick Institutional Biosafety Committee was convened at 12:02 p.m. in the Building 549 Executive Boardroom with the following members in attendance:

Dr. Randall Morin, Chair
Mr. Joseph Kozlovac, Secretary
Dr. Bruce Crise
Dr. Steve Hughes
Dr. Jeanne Herring
Dr. Melinda Hollingshead

Dr. Stephen Creekmore
Dr. Henry Hearn
Dr. Michael Baseler
Dr. Donald Court
Ms. Carol Ingraham Tobias
Mr. Lucien Winegar, Esq.

Members not in attendance: Dr. David Garfinkel, Ms. Cheryl Parrott (Ex Officio)

Others in attendance: Ms. Cara Lamberson, Mr. Tom Danver, Dr. Jinhua Lu, Dr. Steve Giardina, Dr. Andy Hurwitz, Dr. Personal Info

INTRODUCTION

Dr. Morin called the meeting to order.

ACCIDENT REVIEW

Dr. Steve Giardina briefed the IBC on the investigation conducted by BDP into the centrifuge accident that was reported to the committee at the November meeting. Dr. Giardina informed the committee that the BDP, in conjunction with EHS, will develop a training curriculum for individuals utilizing laboratory equipment such as centrifuges, autoclaves, etc. Dr. Giardina also informed the committee that BDP would establish a Safety Committee composed of both supervisory and staff level employees. Mr. Kozlovac has also been invited to serve as a member of this committee that will meet quarterly to discuss training and risk assessment issues for new and on-going projects. Dr. Crise asked about the planning of projects and if "dry runs" would be scheduled when new processes or new equipment in conjunction with infectious material were utilized. Dr. Morin expressed a concern regarding the volume of work that BDP conducted in its existing facilities many of which are marginally adequate for the

planned work. Dr. Creekmore explained to the committee how the Biological Resource Branch (BRB) intensively reviews space planning and project management for all BDP projects.

REVIEW OF PROTOCOLS

Dr. Hollingshead introduced a recombinant DNA registration entitled, *Modulating T cell activation in the anti-tumor and autoimmune responses* submitted by Dr. Andy Hurwitz. Dr. Hollingshead reviewed the particulars of the proposal and informed the committee that one of the issues of concern would be endogenous recombination in the mouse. Dr. Crise asked about the origins of the expression system that is proposed for this work. Dr. Hurwitz informed the committee that he had obtained the expression system from a colleague. Dr. Crise and Dr. Hughes wanted to know the nature of the HIV envelope inactivation (Deletion or Frame Shift). If the envelope inactivation is a result of deletion the IBC would like to know how large the deletion is. Sequencing of this part of the construct would indicate how disabled the viral vector is. (After the meeting Dr. Hurwitz informed Ms. Lamberson via email that the viral vector is a deletion mutant in both HIV vpr and env).

Approval will be granted once the IBC has received and reviewed additional information on the viral vector submitted by the PI.

Dr. Baseler introduced a renewal recombinant DNA registration with significant amendments entitled, *Regulation of the Cell Cycle by cyclin-dependant kinases* submitted by Dr. Philipp Kaldis. Many of the IBC members were concerned due to the lack of clarity in the submission. An example of this is that adenoviral vectors are added into the later part of the registration document but are not mentioned initially, no indication on whether these vectors would be replication competent or not was provided. The registration indicates that no amphotropic retroviral vectors will be utilized and later states that amphotropic vectors may be utilized in certain circumstances. IBC members believed that some fundamental issues in vectorology were missing from this particular submission. Not enough detailed information on inserts was provided but rather broad categories such as viral oncogenes, tumor suppressor genes, etc. Dr. Crise suggested that the PI renew his existing registration and submit a new more detailed registration for the new vectors. Dr. Hughes suggested that the IBC should develop a guidance page on viral vectors that addresses some of the relevant IBC issues of concern for researchers to utilize as a resource that could be placed on the IBC webpage.

Tabled. IBC Secretary will contact PI to have him resubmit renewal registration and submit new registrations to address other viral vector expression. Biosafety officer and various IBC members develop a guidance page on viral vectors.

Dr. Creekmore introduced a pathogen registration entitled, Transformation of RWPE-1 Prostate cell line with PhIP. The IBC would like the PI to further describe the use of carcinogen, how this material will be utilized in the laboratory. The IBC noted that animal work seemed to be an integral part of this series of experiments however no ASP was indicated. Discussion ensued.

Tabled. PI will need to contact ACUC in order to conduct research with animals. PI shall provide more detailed information regarding carcinogen use.

OTHER BUSINESS

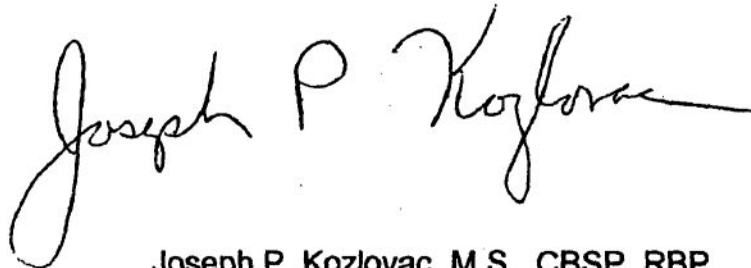
Dr. Morin updated the committee on the 470 demolition, which is essentially complete and the new NIAID BSL-4 facility.

Mr. Kozlovac informed the committee of a minor modification to Dr. Oppenheim's recombinant DNA registration IBC 02-05 to include work with hu and mu EDN (eosinophil derived neurotoxin) and expression vectors containing green fluorescent protein for incorporation into the minutes.

Mr. Kozlovac informed the committee of an amendment to Dr. Ray Harris's pathogen registration P230902JLA01 for incorporation into the minutes. This amendment requested a change in location for production of the chimeric rhino/poliovirus plasmid. The new location is bldg 472, rm 201 for production (fermentation) and rm 203 and 204 for other support functions (centrifugation). The amendment request was reviewed and approved by the BSO, IBC Chair and Dr. Crise (original lead reviewer).

Meeting Adjourned: 1:30 pm

Respectfully submitted,



Joseph P. Kozlovac, M.S., CBSP, RBP
Executive Secretary, NCI-Frederick
Institutional Biosafety Committee

Approved: R. S. Morin

Randall S. Morin, Dr. P.H.
Chairman, NCI-Frederick IBC

xc: Each Committee Member
Dr. Wilttrout
Dr. Reynolds
Mr. Eaton
Dr. Arthur
Mr. Bufter



**NCI-FREDERICK
INSTITUTIONAL BIOSAFETY COMMITTEE**

Minutes – Meeting January 20, 2004
NCI-Frederick

The NCI-Frederick Institutional Biosafety Committee was convened at 12:04 p.m. in the Building 549 Executive Boardroom with the following members in attendance:

Dr. Randall Morin, Chair	Dr. Stephen Creekmore
Mr. Joseph Kozlovac, Secretary	Dr. Henry Hearn
Dr. Bruce Crise	Dr. Michael Baseler
Dr. Steve Hughes	Dr. Donald Court
Dr. Jeanne Herring	Ms. Carol Ingraham Tobias
Dr. David Garfinkel	Mr. Lucien Winegar, Esq.
Ms. Cheryl Parrott (Ex Officio)	

Members not in attendance: Dr. Melinda Hollingshead,

Others in attendance: Ms. Cara Lamberson, Mr. Tom Danver, Dr. Narayan Bhat

INTRODUCTION

Dr. Morin called the meeting to order.

REVIEW OF PROTOCOLS

Dr. Hughes introduced a recombinant DNA registration entitled, *Production of Lentiviral Vectors* submitted by Dr. Naryan Bhat, Gene Expression Laboratory (GEL). Dr. Hughes stated that based upon past reviews and the data provided by Dr. Bhat describing DNA sequence homology between the Invitrogen *Virapower*[™] vectors and HIV, reconstitution of a viable, replication-competent HIV through the process of recombination was not likely. Dr. Crise confirmed Dr. Bhat's analysis and indicated that approximately 1400 base pairs of the HIV *env* gene were missing for the combined total of the vectors. Dr. Hughes posed a discussion-provoking question, that since Dr. Bhat's laboratory is providing a service to other researchers, "Should the IBC provide blanket approval or place restrictions on what type of inserts could be utilized?" Dr. Hughes also expressed concern about work requests in which the requestor places the insert into the vector and provides it to Dr. Bhat for expression. In those cases Dr. Hughes felt it was important that the material be well characterized and that GEL staff understand what they are handling. Dr. Bhat reviewed some of the safety procedures that he will utilize in his laboratories emphasizing that lentiviral

transfections will not take place at the same time as adenoviral transfections, which are also performed in his lab. Dr. Bhat also informed the committee that separate equipment would be utilized for each process. Dr. Garfinkel stated that restrictions placed upon this expression system, such as sequencing to ensure the nature of the insert is consistent with the adenovirus expression system since the GEL utilizes both systems. Dr. Bhat indicated that at present his lab relied heavily on the requestor for verification of the insert, however he would not perform the lentivirus vector work without sequencing first. Mr. Kozlovac suggested that Dr. Bhat build the sequencing cost into his service cost. Dr. Garfinkel stated that this should be done for the adenoviral expression system as well. Dr. Hughes pointed out that when his lab is creating viral stock from plasmids sent to him that he has the material sequenced to ensure they are receiving the correct plasmids. It has been noted by this IBC that when sequencing is required it is often found that the requestor sent the wrong material. Dr. Morin stated that sequencing inserts sent to a lab to ensure that it is the correct material makes for good safety and good science. Dr. Crise, in addition to requiring the GEL to sequence inserts they receive from their customers, wanted to revisit placing a restriction on the insert. Dr. Crise and Dr. Garfinkel suggested that the GEL could not perform work that would involve changing the biosafety of the vector (e.g. reconstitute the lesion in envelope by cloning another envelop gene into the vector system) without coming before the IBC. Dr. Morin suggested that the Biosafety Officer serve as gatekeeper.

Approved.

OLD BUSINESS

Mr. Kozlovac reviewed the amended Kaldis renewal registration that was initially reviewed at the December meeting and was tabled pending a request for more information from the PI. Dr. Hughes stated that he had spoken with Dr. Kaldis however the new submittal does not answer all of his questions. Dr. Crise said that based on the re-submitted document he still felt that there were concerns regarding replication competence. Dr. Morin requested that Mr. Kozlovac provide Dr. Kaldis a list of the IBC's questions and concerns and suggest that he contact Dr. Hughes to discuss the list.

Tabled

Mr. Kozlovac informed the committee that Dr. Whiteside (Transformation of RWPE-1 Prostate cell line with PhIP) had submitted an SOP on how his laboratory would handle work with a carcinogen as requested by the IBC at the December meeting. Mr. Kozlovac stated that he was satisfied with the SOP. Mr. Kozlovac also informed the committee that based upon conversations he had with Dr. Whiteside's supervisor that no animal work was anticipated for the immediate future. If animal work is conducted in the future in conjunction with

this work, Dr. Whiteside shall obtain ACUC approval as well as amend his IBC registration.

Approved

Mr. Kozlovac informed the committee that Dr. Hurwitz (*Modulating T cell activation in the anti-tumor and autoimmune responses*) had submitted his viral vector (deletion mutant in both HIV *vpr* and *env*) for conformational sequencing and once he receives the results he shall forward a copy to the IBC for review.

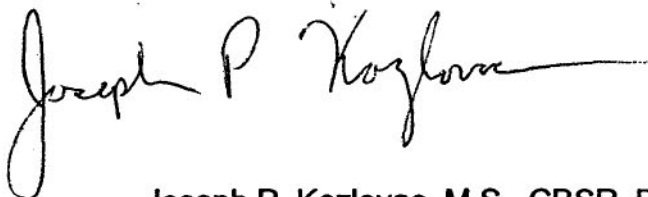
Mr. Kozlovac informed the committee that he had received a written request from Dr. Lu to amend his registration # P190701JLA01 to add another replication defective adenoviral vector, AdCCL-21. AdCCL-21 is a replication defective adenoviral vector expressing human secondary lymphoid chemokine (SLC-CCL-21). Mr. Kozlovac stated that the sequencing data has yet to be provided by Dr. Lu. Committee discussion ensued, and Dr. Creekmore and Dr. Hughes stated that the sequencing data should be provided prior to granting approval.

Approval conditional on IBC receipt of the vector sequencing data.

Dr. Hearn had a question regarding the training curriculum that the BDP had proposed. Mr. Kozlovac informed the committee that he had attended the newly formed BDP Safety Committee and thought that it was a step in the right direction and would lead to enhanced safety awareness for that group. A training course requested by the BDP will cover centrifuge safety and viral vector safety. Cara Lamberson, EHS and Dr. Bruce Crise, AVP will provide the training in sessions to be held on January 27, 28, and 29, 2004.

Meeting Adjourned: 1:20 pm

Respectfully submitted,



Joseph P. Kozlovac, M.S., CBSP, RBP
Executive Secretary, NCI-Frederick
Institutional Biosafety Committee

Approved: 

Randall S. Morin, Dr. P.H.
Chairman, NCI-Frederick IBC

xc: Each Committee Member
Dr. Wiltout

Dr. Reynolds
Mr. Eaton
Dr. Arthur
Mr. Bufter