Whitney, Bruce (NIH/OD) [C]

From: Holeman, Penny [pholeman@lrri.org]
Sent: Friday, September 28, 2007 5:37 PM
To: Whitney, Bruce (NIH/OD) [C]
Subject: LRRI - Incident Report Update

Bruce,

I have attached a cover letter and the minutes as part of the follow-up submission for LRRI's presumed exposure incident.

Please let me know if you need any additional information.

Kind regards,

Penny H. Holeman, MPH, MS, CBSP
Director, Environment, Health & Safety
Director, Biological Safety
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive SE
Albuquerque, NM 87108

Phone (505) 348-9748
Cell (505) 991-1960
Fax (505) 348-9702

Lovelace

September 28, 2007

Bruce Whitney, Ph.D.
Senior Biosafety and Outreach Specialist
NIH Office of Biotechnology Activities
6705 Rockledge Dr., Suite 750
Bethesda, MD 20892-7985

Re: Presumed Exposure Incident Report – Follow Up

Dear Dr. Whitney,

I am submitting a follow-up to the July 26, 2007 incident report submitted on behalf of Lovelace Respiratory Research Institute's (LRRI) Institutional Biosafety Committee (IBC).

The second set of meeting minutes in which the IBC reviewed this incident were approved during the IBC's September 14, 2007 meeting, and are being submitted for your review.

Please feel free to contact me if you have any questions or further information is needed.

Sincerely,

Penny H. Holeman

Penny H. Holeman
Chair, LRRI Institutional Biosafety Committee
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive, S.E.
Albuquerque, New Mexico 87108
(505) 348-9748

PHH/ph
Attachment

LRRI Institutional Biosafety Committee Meeting Minutes
American Home Symposium Center
2425 Ridgecrest Drive SE, Albuquerque, NM 87108
Open Meeting
August 10, 2007
9:45 – 10:25 a.m.

Members in Attendance:
Penny Holeman, Chair
Charles Leonard
Frank Ali
Katherine Gott
Kevin Harrod

Members Absent:
Chuck Hobbs
Richard Conn

Others Present:
Robert Sherwood
Rebecca Sheldon
Amanda Brothers
Denece Kesler

Meeting called to order by Penny Holeman, Chair at 9:45 a.m.

LRRI did not receive any requests from the public to attend the IBC meeting.

I. Review of Minutes

A. July 13th Minutes

Minutes from the July 13, 2007 meeting were reviewed. Dr. Harrod moved to approve the minutes as presented; Frank Ali seconded.

Committee Vote:
Approve: 5 Oppose: 0 Abstain: 0

II. Old Business

A. Notebook Incident

1. Employee Meeting/Training Results
   Ms. Holeman presented the 43 ideas obtained from the first ABSL-3 employee meeting/training session regarding notebook/paper removal. The Committee reviewed the ideas and made the following determinations:

   - Current Practice / No Change Needed – 7 ideas.
   - Add to Corrective Action Plan – 1 idea.
   - On Corrective Action Plan – 16 ideas.

- Implementation Not Recommended – 12 ideas.
- Consider for Future Implementation (related to notebook/paper removal) – 5 ideas.
- Consider for Future Implementation (not related to notebook/paper removal) – 2 ideas.

2. Corrective Action Plan
   Ms. Holeman presented the corrective action plan to the Committee. The Committee unanimously agreed with the plan. Ms. Holeman will continue to update the Committee as the plan is matured and as corrective actions are completed.

   Main Components of the Corrective Action Plan:

   - Review all relevant documents/ training materials to determine if specific language is needed, and revise accordingly.
   - Employee Communications.
   - Review personnel with access to the VHP unloading door, and, if possible, reduce the number of people with access.
   - Develop a monitoring system for items brought in and out of the ABSL-3 Facility.
   - Change ABSL-3 Facility documents to assure they are easily identified.
   - Improve accountability and security of notebooks.
   - Eliminate the need for notebooks associated with anesthesia machine.

III. New Business

A. New Protocol Review

No new rDNA protocols have been submitted to the Committee since the last meeting on July 13, 2007.

IV. Standing Reports

A. Laboratory Inspection Results

Ms. Sheldon informed the committee that there were no findings that required corrective action during her July 31st, 2007 inspection.

B. Laboratory Incidents

Ms. Holeman reported that there have been no laboratory incidents since the last IBC meeting on July 13, 2007.

C. Quarterly Select Agent Inventory Results

The quarterly inventory is not yet due.

V. Other Business

There was no other business.

The next meeting of the LRRI Institutional Biosafety Committee is scheduled on September 14, 2007.

The meeting adjourned at 10:25 a.m.

Respectfully submitted,

Amanda Brothers, Recording Secretary

9/14/07
Date

Whitney, Bruce (NIH/OD) [C]

From: Holeman, Penny [pholeman@lrri.org]
Sent: Monday, August 13, 2007 4:38 PM
To: Whitney, Bruce (NIH/OD) [C]
Subject: RE: LRRI - Incident Report Update

Bruce,

There will be more coming next month, as the IBC conducted a methodical review of the employee suggestions and the Corrective Action Plan during last Friday's meeting. We have started implementing corrective actions, and have closed some out, but Friday was the Committee's first methodical review. We will need to approve these minutes at our next meeting before they are considered to be a final document. Some of our corrective actions will take longer to fully implement, so it may be a while before we consider this to be closed out.

Kind regards,

Penny H. Holeman, MPH, MS, CBSP
Director, Environment, Health & Safety
Director, Biological Safety
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive SE
Albuquerque, NM 87108

Phone (505) 348-9748
Cell (505) 991-1960
Fax (505) 348-9702

From: Whitney, Bruce (NIH/OD) [C] [mailto:whitneyb@mail.nih.gov]
Sent: Monday, August 13, 2007 12:01 PM
To: Holeman, Penny
Subject: RE: LRRI - Incident Report Update

Penny,

Thank you for the cover letter and IBC minutes. We will reply to you after we have review all the material.

Regards,

Bruce

Bruce Whitney, Ph.D.
Senior Biosafety and Outreach Specialist (contractor)
NIH Office of Biotechnology Activities
6705 Rockledge Drive, Suite 750
Bethesda, Maryland 20892-7985
Tel: (301) 435-2149
Fax: (301) 496-9839

-----Original Message-----
From: Holeman, Penny [mailto:pholeman@lrri.org]
Sent: Monday, August 13, 2007 1:54 PM

To: Whitney, Bruce (NIH/OD) [C]
Subject: LRRI - Incident Report Update

Bruce,

I have attached a cover letter and a copy of the minutes in which the presumed exposure incident was discussed.

Kind regards,

Penny H. Holeman, MPH, MS, CBSP
Director, Environment, Health & Safety
Director, Biological Safety
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive SE
Albuquerque, NM 87108

Phone (505) 348-9748
Cell (505) 991-1960
Fax (505) 348-9702

Whitney, Bruce (NIH/OD) [C]

From: Holeman, Penny [pholeman@lrri.org]
Sent: Monday, August 13, 2007 1:54 PM
To: Whitney, Bruce (NIH/OD) [C]
Subject: LRRI - Incident Report Update

Bruce,

I have attached a cover letter and a copy of the minutes in which the presumed exposure incident was discussed.

Kind regards,

Penny H. Holeman, MPH, MS, CBSP
Director, Environment, Health & Safety
Director, Biological Safety
Lovelace Respiratory Research Institute
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Fax (505) 348-9702

Lovelace

August 13, 2007

Bruce Whitney, Ph.D.
Senior Biosafety and Outreach Specialist
NIH Office of Biotechnology Activities
6705 Rockledge Dr., Suite 750
Bethesda, MD 20892-7985

Re: Presumed Exposure Incident Report – Follow Up

Dear Dr. Whitney,

I am submitting a follow-up to the July 23, 2007 incident report submitted on behalf of Lovelace Respiratory Research Institute's (LRRI) Institutional Biosafety Committee (IBC).

The minutes of the meeting in which the IBC reviewed this incident were approved during the IBC's August 10, 2007 meeting, and are being submitted for your review.

Please feel free to contact me if you have any questions or further information is needed.

Sincerely,

Penny H. Holeman

Penny H. Holeman
Chair, LRRI Institutional Biosafety Committee
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive, S.E.
Albuquerque, New Mexico 87108
(505) 348-9748

PHH/ph
Attachment

LRRI Institutional Biosafety Committee Meeting Minutes
American Home Symposium Center
2425 Ridgecrest Drive SE, Albuquerque, NM 87108
Open Meeting
July 13, 2007
9:50 – 10:07 a.m.

Members in Attendance:
Penny Holeman, Chair
Charles Leonard
Richard Conn
Katherine Gott
Kevin Harrod

Members Absent:
Chuck Hobbs
Frank Ali

Others Present:
Robert Sherwood
Rebecca Sheldon
Amanda Brothers
Patricia Shuman

Meeting called to order by Penny Holeman, Chair at 9:50 a.m.

LRRI did not receive any requests from the public to attend the IBC meeting.

I. Review of Minutes

A. March 9, 2007 Minutes

Minutes from the April 13, 2007 meeting were reviewed. Dr. Harrod moved to approve the minutes as presented; Dr. Conn seconded.

Committee Vote:
Approve: 5 Oppose: 0 Abstain: 0

II. Old Business

A. Communication from OBA

Ms. Holeman updated the Committee about the Sunshine Project's complaint to OBA. The Sunshine Project alleged that LRRI failed to comply with the NIH Guidelines by not providing minutes of its IBC meetings. OBA sent a response letter to LRRI and the Sunshine Project stating that LRRI's response seems consistent with OBA's guidelines, and that OBA would not be pursuing this matter further.

B. CDC Registration

Ms. Holeman informed the committee that the Centers for Disease Control and Prevention (CDC) renewed LRRI's registration and that LRRI is authorized to possess, use and transfer Select Agents and Toxins until 2010.

III. New Business

A. New Protocol Review

No new rDNA protocols have been submitted to the Committee since the last meeting on April 13, 2007.

B. Quarterly Select Agent Inventory Results

Ms. Holeman reported that all Select Agents were accounted for in the second quarter inventory.

C. Laboratory Inspection Results

Ms. Sheldon informed the Committee that no findings were associated with the rooms in which the rDNA study was conducted.

D. Notebook Incident

Ms. Holeman described an incident presumed to involve the three recombinant strains of *B. anthracis*.

LRRI believes that the notebook involved may never have been contaminated with *B. anthracis*, however, LRRI reported this incident to the appropriate agencies because the notebook was in a room where *B. anthracis* was used, and the notebook was not properly decontaminated.

Incident Description

On June 5, 2007 an employee informed the BSO that she inadvertently removed a notebook from an ABSL-3 containment facility on May 31, 2007. The notebook is smaller than the standard size (approx. 8" x 4"), was hung by a chain from an anesthesia machine, and was not necessarily very noticeable. The machine was used in a room where a study with *B. anthracis* was conducted. On July 10, 2007 the BSO learned that this study also involved the three recombinant strains of *B. anthracis* and immediately reported the incident to OBA. (The three recombinant strains each had a specific gene removed. When the Committee reviewed and approved this protocol during a previous meeting, these strains were believed to have a reduced function when compared with the standard strain.)

The room had been successfully decontaminated using vaporized hydrogen peroxide (VHP) while the anesthesia machine and notebook were still in the room. The room decontamination was verified with spore strips that were placed at intervals around the room. The room had been decontaminated as part of a larger facility decontamination for annual preventive maintenance and equipment certification.

Although a document published by the manufacturer of the VHP equipment indicates that VHP can successfully surface decontaminate cellulose (paper), LRRI has taken the conservative position that the paper in the notebook might

have contained viable organisms because paper can interfere with the VHP process.

The employee removed the anesthesia machine (and notebook) from the containment facility, where it was retrieved by a non-containment employee. The first employee was wearing gloves (as was part of LRRI's 'stand down' procedure after the facility was decontaminated) and did not have an identified exposure incident. The second employee leafed through the notebook with his bare hands to evaluate data entry, and transferred the machine and notebook to the locked calibration laboratory. The second employee handled the notebook with intact skin, and washed his hands afterward for a very short period of time. LRRI has no knowledge that anyone else touched the anesthesia machine or notebook.

The events occurred on a contiguous piece of property located in a remote section of the Air Force base. Other LRRI employees, the public, and the environment were determined to have no risk of exposure.

Immediate Actions

The first employee was compliant with her vaccination schedule. She did not have an identified exposure incident, no medical evaluation or treatment was provided, and she has remained asymptomatic. The first employee stated that she was so focused on removing the anesthesia machine from the facility, she had forgotten that the notebook was hanging from the machine.

The second employee was not vaccinated prior to the incident, and was asymptomatic for anthrax infection on June 5, 2007. He was immediately referred to Occupational Health for medical evaluation and treatment - even though the BSO is unaware of anyone becoming infected by direct contact with potentially contaminated paper in a laboratory setting. The treating physician prescribed Ciprofloxacin for 10 days. The employee was compliant with his medication, and has not experienced any symptoms of anthrax illness.

The notebook was bagged, and was placed in a running Class II BSC back inside the containment facility. The surfaces that the second employee might have contaminated as he transported the anesthesia machine and notebook to the calibration laboratory were chemically decontaminated. The incident was immediately reported to CDC on June 5, 2007.

Follow Up Actions

The incident was reported to the New Mexico Public Health Department, and a Form 3 was submitted to CDC.

Swab samples taken from the notebook yielded bacterial and fungal growth, but there were no indications of *B. anthracis* growth.

Signs were posted inside the containment facility at locations where items exit the facility. These signs clearly stated that paper and notebooks are not to be removed from the facility.

Four documents were reviewed including the LRRI Biological Safety Manual and facility-specific standard operating procedures. These documents will be revised to more clearly state that paper/notebooks shall not be removed from the facility without first being autoclaved.

Meetings were held by key members of management, and another meeting was held for all employees who work in the containment facility. The focus of these meetings was to: 1) emphasize that notebooks are not to leave the containment facility, 2) reinforce the current procedure that paper/data shall be scanned out of the facility, and 3) identify corrective actions that will prevent a similar event from happening in the future.

The proposed corrective actions are being reviewed, and an implementation plan is being developed for the Committee's review.

A formal report (to follow up on the immediate report) to OBA will need to be filed.

IV. Other Business

There was no other business

The next meeting of the LRRI Institutional Biosafety Committee is scheduled on August 10, 2007.

The meeting adjourned at 10:07 a.m.

Respectfully submitted,

Amanda Brothers
Amanda Brothers, Recording Secretary

8/10/07
Date

Whitney, Bruce (NIH/OD) [C]

From: Holeman, Penny [pholeman@lrri.org]
Sent: Thursday, July 26, 2007 5:42 PM
To: Whitney, Bruce (NIH/OD) [C]
Subject: LRRI Incident Report

Bruce,

I have attached LRRI's incident report, a document regarding VHP surface decontamination of cellulose, and the IBC minutes of the meeting in which the protocol was reviewed. Please let me know if OBA requires any additional information.

Kind regards,

Penny H. Holeman, MPH, MS, CBSP
Director, Environment, Health & Safety
Director, Biological Safety
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive SE
Albuquerque, NM 87108

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Cell (505) 991-1960
Fax (505) 348-9702

Lovelace

July 26, 2007

Bruce Whitney, Ph.D.
Senior Biosafety and Outreach Specialist
NIH Office of Biotechnology Activities
6705 Rockledge Dr., Suite 750
Bethesda, MD 20892-7985

Re: Presumed Exposure Incident Report

Dear Dr. Whitney,

I am submitting an incident report on behalf of Lovelace Respiratory Research Institute's (LRRI) Institutional Biosafety Committee (IBC). This report is being submitted within 15 working days of the immediate report to OBA that was e-mailed to you on July 10, 2007.

At the time the incident was recognized, the BSO was aware that there was a presumed exposure incident involving *B. anthracis*. On July 10th, the BSO learned that this presumed exposure incident may also have involved three recombinant strains of *B. anthracis*.

LRRI believes that the notebook involved may never have been contaminated with *B. anthracis*, however, LRRI is reporting this incident because the notebook was in a room where *B. anthracis* was used, and the notebook was not properly decontaminated.

Incident Description

On June 5, 2007 an employee informed the BSO that she inadvertently removed a notebook from an ABSL-3 containment facility on May 31, 2007.

The notebook is smaller than the standard size (approx. 8" x 4"), was hung by a chain from an anesthesia machine, and was not necessarily very noticeable. The machine was used in a room where a study with *B. anthracis* and three recombinant strains of *B. anthracis* was conducted. (The three recombinant strains each had a specific gene removed. When the Institutional Biosafety Committee reviewed this protocol, these strains were thought to have a reduced function when compared with the standard strain.)

The room had been successfully decontaminated using vaporized hydrogen peroxide (VHP) while the anesthesia machine (and notebook) was still in the room. The room

(Presumed Exposure Incident Report, Cont.)

decontamination was verified with spore strips that were placed at intervals around the room. The room had been decontaminated as part of a larger facility decontamination for annual preventive maintenance and equipment certification.

Although the attached document indicates VHP can successfully surface decontaminate cellulose (paper), LRRI took the conservative position that the paper in the notebook might have contained viable organisms because paper can interfere with the VHP process. The employee removed the anesthesia machine (and notebook) from the containment facility, where it was retrieved by a non-containment employee.

The first employee was wearing gloves (as was part of LRRI's 'stand down' procedure after the facility was decontaminated) and did not have an identified exposure incident. The second employee leafed through the notebook with his bare hands to evaluate data entry, and transferred the machine and notebook to the locked calibration laboratory. The second employee handled the notebook with intact skin, and washed his hands afterward for a very short period of time. LRRI has no knowledge that anyone else touched the anesthesia machine or notebook. The events occurred on a contiguous piece of property located in a remote section of an Air Force base.

Other LRRI employees, the public, and the environment were determined to have no risk of exposure.

Immediate Response

The first employee was compliant with her vaccination schedule. She did not have an identified exposure incident, no medical evaluation or treatment was provided, and she has remained asymptomatic. The first employee stated that she was so focused on removing the anesthesia machine from the facility, she had forgotten that the notebook was hanging from the machine.

The second employee was not vaccinated prior to the incident, and was asymptomatic for anthrax infection on June 5, 2007. He was immediately referred to Occupational Health for medical evaluation and treatment - even though the BSO is unaware of anyone becoming infected by direct contact with potentially contaminated paper in a laboratory setting. The treating physician prescribed Ciprofloxacin for 10 days. The employee was compliant with his medication, and has not experienced any symptoms of anthrax illness.

The notebook was bagged, and was placed in a running Class II BSC back inside the containment facility. The surfaces that the second employee might have contaminated as he transported the anesthesia machine and notebook to the calibration laboratory were chemically decontaminated. The incident was reported to the Centers for Disease Control and Prevention, and to the New Mexico Public Health Department.

(Presumed Exposure Incident Report, Cont.)

Subsequent Response

Swab samples taken from the notebook yielded bacterial and fungal growth, but there were no indications of *B. anthracis* growth.

Signs were posted inside the containment facility at locations where items exit the facility. These signs clearly stated that paper and notebooks are not to be removed from the facility.

Four documents were reviewed including the LRRI Biological Safety Manual and facility-specific standard operating procedures. These documents will be revised to more clearly state that paper/notebooks shall not be removed from the facility without first being autoclaved.

Meetings were held by key members of management, and another meeting was held for all employees who work in the containment facility. The focus of these meetings was to: 1) emphasize that notebooks are not to leave the containment facility, 2) reinforce the current procedure that paper/data shall be scanned out of the facility, and 3) identify corrective actions that will prevent a similar event from happening in the future. The proposed corrective actions are being reviewed, and an implementation plan is being developed.

The incident was reported to the IBC, and it was discussed at the next IBC meeting. A copy of the IBC minutes in which the study protocol was initially reviewed is attached. The minutes of the meeting in which the IBC reviewed this incident have not been finalized, and will be submitted to OBA when they have been approved.

Please feel free to contact me if you have any questions or further information is needed.

Sincerely,

Penny H. Holeman

Penny H. Holeman
Chair, LRRI Institutional Biosafety Committee
Lovelace Respiratory Research Institute
2425 Ridgecrest Drive, S.E.
Albuquerque, New Mexico 87108
(505) 348-9748

PHH/ph
Attachments

VAPORIZED HYDROGEN PEROXIDE APPLICATION FOR ANIMAL FEEDBAG SURFACE DECONTAMINATION

INTRODUCTION

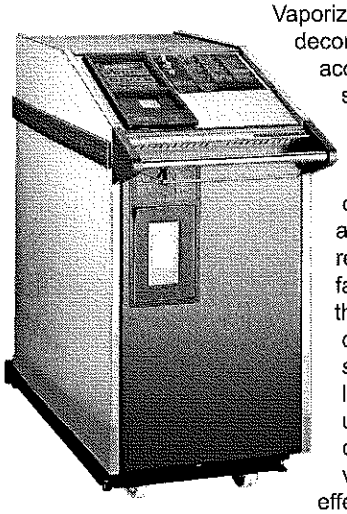

Figure 1. VHP Mobile Biodecontamination System

Vaporized Hydrogen Peroxide (VHP®) decontamination has gained wide acceptance in multiple facets of science and industry. The pharmaceutical and biotechnology industries are now applying this mode of decontamination for many uses and applications. Animal research and production facilities are increasingly using this technology in room decontamination, device sterilization and processing lines because of its ease of use, low health/safety concerns, ease of process validation, time and cost effectiveness. A major application that is consistently gaining wide acceptance in animal facilities is the VHP pass-through process. In this process, a facility would possess a receiving (unclean) area/room to process equipment and material, followed by a room for VHP decontamination and a post decontamination clean area/room. In this process, facilities that must have an environment free of animal pathogens as well as other microorganisms are assured of not contaminating an environmentally controlled facility. VHP decontamination has a broad spectrum of efficacy against micro-organisms[1-4]. VHP technology has a wide array of material compatibility including many metal and plastic alloys (Table 1.) In addition to entire room decontamination applications, common equipment processed in animal facilities includes cages, racks, bedding, food containers and support devices. The most challenging material component is cellulose based due to the possible absorbance of hydrogen peroxide. Although cellulose based materials pose a challenge in some instances of application, with proper cycle development and aeration, these too can be decontaminated using Vaporized Hydrogen Peroxide. In this study, multi-layered cellulose poultry feedbags were processed using typical VHP 1000 cycles for surface decontamination. In simulating a decontamination pass-through process, bag samples were inoculated with *Bacillus stearothermophilus* (ATCC#7953) spores at a population of 106 and exposed to various VHP cycles for optimization. *Bacillus stearothermophilus* spores were utilized because of their acceptance as the most resistant organism to Vaporized Hydrogen Peroxide[5]. Uninoculated material samples were also exposed during these cycles and tested for residuals of hydrogen peroxide. Qualitative penetration studies were performed using VHP Chemical Indicators between the various layers and the interior of a resealed feedbag for the presence of hydrogen peroxide.

Table 1. Examples of Material Compatibility with Vaporized Hydrogen Peroxide

Material	Compatibility
Aluminum, Titanium, Stainless Steel	Excellent
Anodized Aluminum, Steel, Brass, Copper	Good
Polyethylene, Polypropylene, Tefzel, CPVC	
Polystyrene, Polyvinyl Chloride, PTFE, PES	Excellent
Polymethylpentene, PVDF, PEEK, Kel-F, Aflas	
Polyacetal, PPO, Polysulfone, Ultem, ABS, PMMA	Excellent
PolyethyleneTerephthalate (PET)	Good
Silicon Rubbers, Kelrez	Excellent
Glass	Excellent
Butyl Rubber, ChemRaz	Good

Source: STERIS Corporation, Mentor, OH

MATERIALS & METHODS

Feedbag Samples and Source: The investigative material was a Buckeye Nutrition Poultry Feedbag from Buckeye Feed Mills, Inc., Dalton, Ohio. This three-layer cellulose bag is rated as a freight-shipping bag for 50 pounds by its manufacturer, Ray C. Sprosty Bag Company, Wooster, Ohio. Samples to be inoculated with spore suspension were cut into 2cm x 8cm strips. Blank samples were cut into 1cm x 10cm strips to determine residuals.

Spore Suspension Preparation and Inoculation: *Bacillus stearothermophilus* (ATCC#7953) spore suspension was prepared by STERIS Biological Operations. Suspension population was verified at 106/20ul through serial dilutions and counts of colony forming units (CFUs) on tryptic soy agar (Lot no. 070901B). Sample strips were inoculated by micropipette with 20ul of spore suspension, allowed to dry and the mean population of strip samples was determined to be 1.3 x 106 through the counting of CFUs.

Cycle Development and Exposures: To develop a VHP pass-through decontamination cycle, a VHP 1000 generator (Figure 1.) (SN:0114699009) loaded with a 31% H_2O_2 cartridge (Lot PEO11A) was connected to a 22 ft^3 La Calhene Flexible isolator (Model MNI56143). Installed within the isolator was a FLUKE Hydra II (Model 7510002) to monitor temperature and humidity. Also installed inside the isolator to monitor hydrogen peroxide and water concentrations was a UOP Guided Wave H_2O_2 monitor (Model 1056). The cycles were programmed and performed in succession to optimize a six log reduction on inoculated samples for verification of surface decontamination as shown in Table 2. Five inoculated sample strips were inserted onto a stainless steel coil and exposed for each cycle. Uninoculated strips were exposed to cycle three parameters only.

Table 2. Experimental VHP 1000 Cycle Parameters

Phase	Cycle 1	Cycle 2	Cycle 3
Dehumidification			
Air Return	18 SCFM	18 SCFM	18 SCFM
Time	20 minutes	20 minutes	20 minutes
Absolute Humidity	4.6 %	4.6 %	4.6 %
Conditioning			
Air Return	10 SCFM	10 SCFM	10 SCFM
Time	2 minutes	2 minutes	2 minutes
Injection Rate	2.3 g/minute	2.6 g/minute	2.6 g/minute
Sterilization			
Air Return	10 SCFM	10 SCFM	10 SCFM
Time	60 minutes	45 minutes	30 minutes
Injection Rate	1.8 g/minute	1.8 g/minute	1.8 g/minute
Aeration			
Air Return	18 SCFM	18 SCFM	18 SCFM
Time	30 minutes	30 minutes	45 minutes

Inoculated and uninoculated samples were exposed during the entire cycle times. Hydrogen peroxide concentrations were monitored during all test cycles via the UOP sensor probe within the isolator.

Sample Culturing: Immediately following the aeration phase of each cycle, exposed inoculated strips were aseptically removed from the isolator and cultured in tryptic soy broth (Lot 210801). Cultured samples were then incubated at 58°-59°C in a HotPack Incubator (Model 317522) for seven days. Daily growth checks for media turbidity were made.

Hydrogen Peroxide Residual Assay: Test strips were exposed to cycle three parameters and were tested for residuals 24 hours after exposure. Square centimeter samples of exposed and unexposed (control) strips were tested for H_2O_2 residual using a Xylenol Orange Assay. Extractions were performed at 3, 5, 10 ml for exposed samples and unexposed controls were performed at 3 ml. At each extraction five samples were tested.

VHP Penetration Assay: A sample poultry feedbag was cut from the top and emptied of its contents, five VHP Chemical Indicators (STERIS NB305 lot 204506) were placed between the outer and middle layer, the middle and interior layer and inside the feedbag. The feedbag top was then folded and resealed with clear STA brand packing tape. Five chemical indicators were taped to the exterior of the feedbag for control purposes. These indicators were then exposed to cycle three conditions and retrieved from the feedbag after exposure for interpretation.

RESULTS:

Inoculated Sample Exposures: Cycles were performed 24 hours after each other to interpret 24 hour culture reads. If the previous cycle exhibited no growth in 24 hours, the sterilization phase time of the decontamination cycle was decreased incrementally. Following exposure to the various test cycles and incubation, all inoculated test samples exhibited no growth after seven days (Table 3).

Table 3. Inoculated Sample Results after Seven-Day Incubation

Cycle	Sterilization Phase Time	Mean H_2O_2 Concentration	# Positive/ # Exposed	Log Reduction
1	60 minutes	1.5mg/L	0 / 5	Six
2	45 minutes	1.5mg/L	0 / 5	Six
3	30 minutes	1.5mg/L	0 / 5	Six

Hydrogen Peroxide Residuals: Uninoculated samples were exposed to cycle three parameters and were tested 24 hours after exposure for hydrogen peroxide residuals. The results (Table 4.) of surface residuals are reported in ug/cm^2. Three different dilutions are standard testing procedure for testing residuals.

Table 4. Hydrogen Peroxide Residuals (ug/cm^2)

Extractions	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Mean Residual
Control	.077	.061	.074	.068	.090	.074
3ml Extraction	.068	.075	.070	.073	.068	.071
5ml Extraction	.075	.060	.080	.073	.080	.074
10ml Extraction	.093	.106	.113	.101	.070	.097

Vaporized Hydrogen Peroxide Penetration: Chemical indicators were retrieved after exposure to cycle three parameters from each layer. The mean H_2O_2 concentration for the sterilization phase was 1.5 mg/L inside the isolator. The five chemical indicators located between the outer and middle layers of the feedbag showed very faint discoloration from the control color block. The five chemical indicators from between the middle and inner layer showed no discoloration from the control color block. The five chemical indicators from the interior of the feedbag showed no discoloration from the control color block. The exterior bag chemical indicators all strongly tested positive for the presence of Vaporized Hydrogen Peroxide.

Table 5. VHP Chemical Indicator Presence of Hydrogen Peroxide

Chemical Indicator Location	Presence of Vaporized Hydrogen Peroxide
Exterior	Strong Presence
Between outer and middle layer	Slight Presence
Between middle and inner layer	None
Interior	None

DISCUSSION:

The exposed sample cultures clearly demonstrated a bioburden reduction that gives a level of decontamination warranted for applications in critical environment animal research and housing facilities. The optimization of VHP 1000 generator cycles in this study is intended to demonstrate the process of cycle development and the adaptability to decontaminate materials that may pose a challenge with other modes of decontamination. Residuals from the exposure to the VHP decontamination cycle are below safety limits and within 24 hours, quite comparable to control samples that were not exposed to Vaporized Hydrogen Peroxide. After exposure of an entire poultry feedbag during the penetration study, the exterior of the bag showed no visible signs of ink print bleaching, fading or running on the surface. The integrity of the exterior layer showed no visible cracking or splitting. There was no presence of rigidity or brittleness on the exterior layer. Odor and texture were comparable to a control bag that was not exposed.

The multi-layers of the feedbag did not allow penetration to the interior and there are no results suggesting the bag contents integrity would be affected by Vaporized Hydrogen Peroxide application. Chemical indicators were evaluated strictly as a qualitative test and were not to be interpreted as quantifying the presence of hydrogen peroxide.

CONCLUSIONS: The anti-microbial efficacy of a VHP 1000 biodecontamination cycle in this material application can successfully reduce surface contaminants on feedbags. Proper cycle development and aeration can overcome exposure material challenges in most cases where absorbance and decomposition could pose difficulties.

The advantages to this type of decontamination system are:

1) Assurance of high-level sporicidal, bactericidal, viricidal and fungicidal efficacy.
2) The process is adaptable and easily controlled.
3) The process can be easily validated to meet user needs.
4) The sterilant decomposes to environmentally friendly products.
5) Personnel safety and health issues are minimized because of containment.

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LRRI Institutional Biosafety Committee Meeting Minutes
American Home Symposium Center
2425 Ridgecrest Drive SE, Albuquerque, NM 87108
Closed Meeting
March 9, 2007 8:35– 9:25 a.m.

Members in Attendance:
Penny Holeman, Chair
Chuck Hobbs
Charles Leonard
Richard Conn
Katherine Gott
Frank Ali
Kevin Harrod

Members Absent:

Others Present:
Amanda Brothers

Meeting called to order by Penny Holeman, Chair at 8:35 a.m.

LRRI did not receive any requests from the public to attend the IBC meeting.

I. Review of Minutes

A. May 12, 2006 Minutes

Minutes from the May 12, 2006 meeting were reviewed. Dr. Hobbs moved to approve the minutes; Dr. Leonard seconded. Frank Ali abstained from the vote since he was not an IBC member for the May 12th meeting.

Committee Vote: Approve: 6 Oppose: 0 Abstain: 1

B. October 27, 2006 Minutes

Minutes from the October 27, 2006 meeting were reviewed. Dr. Conn moved to approve the minutes; Frank Ali seconded.

Committee Vote: Approve: 7 Oppose: 0 Abstain: 0

C. February 9, 2007

Minutes from the February 9, 2007 meeting were reviewed. Dr. Hobbs moved to approve the minutes; Dr. Harrod seconded.

Committee Vote: Approve: 7 Oppose: 0 Abstain: 0

II. Old Business

A. The Sunshine Project Communication

On February 15, 2007, Ms. Holeman made a second attempt to send LRRI's November 3, 2006 response to The Sunshine Project. The letter was faxed, and was also sent by certified mail, return receipt request with restricted delivery.

The Sunshine Project's Edward Hammond sent Ms. Holeman an e-mail stating that the fee LRRI requests, to partly off-set the costs of providing IBC minutes, is excessive.

B. Biological Safety Manual Revision

Ms. Holeman sent the draft revisions to the Committee members for review and comment in advance of the meeting. The purpose of this revision was to align the containment criteria for BSL-1, BSL-2 and BSL-3 with the recently published 5th Edition of the CDC/NIH document titled "Biosafety in Microbiological and Biomedical Laboratories". The revision also includes the containment criteria in the NIH guidelines.

Dr. Leonard moved to approve the proposed revisions; Frank Ali seconded.

Committee Vote:
Approve: 7 Oppose: 0 Abstain: 0

III. New Business

The IBC received its first protocol for review.

A. New Protocol Review

1. ES&H Project "Pilot Study of Anthrax Infection in following Bronchial Instillation of the Ames Strain of Three Mutant Forms of *B. anthracis*".

   Principal Investigator:

In this study, will be exposed to four strains of *Bacillus anthracis*, three of which are genetically engineered. The information gained from this study is believed to lead to better therapies and better design of a vaccine.

Agent Characteristics: The three genetically engineered strains were developed at another institution. Each of the genetically engineered strains has had one gene removed and replaced with a kanamycin-resistance gene. The genes that were removed code for edema factor, lethal factor, and an antigen that protects the bacteria. It is anticipated that these strains will have a reduced function when compared with the standard strain in this animal model, but they are still expected to be pathogenic at high doses.

Types of manipulations planned:

1. Grow spores (TSB medium at 37°C for 4 days)
2. Test titer (dilute in sterile 1% peptone and plate on TSA)
3. Test for percent spore (heat shock for 30 min at 65°C and plate on TSA)
4. Inject suspension into the trachea of the animal using a catheter or Bronchoscope.

5. Titer suspension on TSA plates
6. Recover blood and quantitate viable *B. anthracis*
7. Recover tissue at necropsy and quantitate viable *B. anthracis*

Source(s) of inserted DNA sequences: Plasmid

Nature of inserted DNA sequences: The omega-kanamycin cassette contains kanamycin-resistance gene used as a marker. Kanamycin is not the antibiotic of choice for treatment of infection in humans.

Hosts(s) and vector(s) to be used:

Expression of foreign gene: kanamycin resistance *in vitro*

Containment conditions: BSL-3 containment for *in vitro* work and ABSL-3 containment for *in vivo* work

Applicable section of the NIH Guidelines: Section III-D-4-b

ES&H is to verify that all study personnel have completed all training requirements before they begin work on this study.

Dr. Harrod moved to approve this protocol; Frank Ali seconded.

Committee Vote:
Approve: 7 Oppose: 0 Abstain: 0

B. Communications from OBA

Ms. Holeman discussed the letter LRRI received from OBA regarding The Sunshine Project's complaint of LRRI not responding to their 2006 request for minutes. Ms. Holeman responded to OBA on February 28, 2007. LRRI is awaiting a response.

Ms. Holeman distributed and discussed the most recent guidance document on the content of Institutional Biosafety Committee Meeting Minutes. The Committee was in agreement as to what is expected out of LRRI's IBC Minutes.

The next meeting of the LRRI Institutional Biosafety Committee is scheduled on April 13, 2007. The meeting adjourned at 9:25 a.m.

Respectfully submitted,

Amanda Brothers

Amanda Brothers, Recording Secretary

4/25/07

Date

Whitney, Bruce (NIH/OD) [C]

From: Holeman, Penny [pholeman@lrri.org]

Sent: Tuesday, July 10, 2007 6:33 PM

To: Whitney, Bruce (NIH/OD) [C]

Subject: Immediate Report of a Presumed Exposure to Organisms Containing Recombinant DNA Molecules

Dr. Whitney,

To follow up on our telephone conversation earlier today, LRRI is filing an immediate report of an accident that resulted in a presumed exposure to organisms containing recombinant DNA molecules. At the time the incident was recognized, the BSO was aware that there was a presumed exposure incident involving *B. anthracis.* Today, the BSO learned that this presumed exposure incident may also have involved three recombinant strains of *B. anthracis.*

Incident Description

On June 5, 2007 an employee informed the BSO that she inadvertently removed a notebook from an ABSL-3 containment facility on May 31, 2007. The notebook is smaller than the standard size (approx. 8" x 4"), and was hung by a chain from an anesthesia machine. The machine was used in a room where a study with *B. anthracis* and three recombinant strains of *B. anthracis* was conducted. (The three recombinant strains each had a specific gene removed. When the Institutional Biosafety Committee reviewed this protocol, these strains were thought to have a reduced function when compared with the standard strain.) The room had been successfully decontaminated using vaporized hydrogen peroxide (VHP). The room decontamination was verified with spore strips placed at intervals around the room. The room had been decontaminated as part of a larger facility decontamination for annual preventive maintenance and equipment certification.

Although there is at least one report indicating VHP can successfully decontaminate paper, LRRI presumed the notebook was contaminated because paper is also known to interfere with the VHP process. The employee removed the anesthesia machine (with the attached notebook) from the containment facility, where it was retrieved by a non-containment employee. The first employee was wearing gloves (as was part of LRRI's 'stand down' procedure after the facility decontamination) and did not have an identified exposure incident. The second employee leafed through the notebook to evaluate data entry, and transferred the machine and notebook to the locked calibration laboratory. The second employee handled the notebook with intact skin, and washed his hands afterward for a very short period of time. LRRI has no knowledge that anyone else touched the anesthesia machine or notebook. The events occurred on a contiguous piece of property located in a remote section of an Air Force base. Other LRRI employees, the public, and the environment were determined to have no risk of exposure.

Immediate Response

The first employee was compliant with her vaccination schedule. She did not have an identified exposure incident, no medical evaluation or treatment was provided, and she has remained asymptomatic. The second employee was not vaccinated prior to the incident, and was asymptomatic for anthrax infection on June 5, 2007. He was immediately referred to Occupational Health for medical evaluation and treatment even though the BSO is unaware of anyone becoming infected by contact with potentially contaminated paper. The treating physician prescribed Ciprofloxacin for 10 days. The employee was compliant with his medication, and has not experienced any symptoms of anthrax illness. The notebook was bagged, and was placed in a running Class II BSC inside the containment facility. The surfaces that the second employee might have contaminated as he transported the anesthesia machine and notebook to the calibration laboratory were chemically decontaminated. The incident was reported to the Centers for Disease Control and Prevention, and to the New Mexico Public Health Department.

Swab samples taken from the notebook yielded bacterial and fungal growth, but there were no indications of *B. anthracis* growth.

LRRI will provide the NIH with additional information, including corrective actions in a subsequent report.

Please feel free to contact me if you need any additional information.

Kind regards,

Penny H. Holeman, MPH, MS, CBSP
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Director, Biological Safety
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