How you use our rack is up to you but we guarantee that each ISOcage will maintain a stable, lasting hermetic seal both on and off the rack. We also deliver each rack with a UPS battery back-up so it can be used independently of your power source.

Since safety is the #1 driver of bio-containment, our engineers designed HEPA filtration into this system at the cage-level. This makes personnel safety convenient, flexible and cost-effective since different studies can now be housed in the same rack and room.

We began by designing around the benefits of a premier biocontainment system that maintains negative pressure when the rack is undocked. Our engineers didn’t stop until we could offer you a better night’s sleep knowing that you made the most cost-effective choice in biocontainment.

Our ISOcage System comes to you with an unparalleled pedigree because each one is built with the insights and expertise of leading researchers and a world leader in equipment manufacturing.

The Tecniplast system combines an ISOcage of incomparable durability and design with a biosafety station offering the latest features in personnel protection.

Our system stands out—and it will stand the test of time because our quality control measures are the most stringent in the industry.

The ISOcage System has all the elements of excellence that go into a Tecniplast product so you can be confident it has everything you need to keep your people and facility safe. To learn more visit us on the web at www.tecniplastusa.com or call 877-669-2243.
President's Page

Articles

Comparative Analysis of the Fourth and Fifth Editions of Biosafety in Microbiological and Biomedical Laboratories Section IV (BSL2-4)
Charles J. Crews and Edward E. Gaunt ................................................................. 6

Inactivation of Francisella tularensis Schu S4 in a Biological Safety Cabinet Using Hydrogen Peroxide Fumigation
James V. Rogers and Young W. Choi ....................................................................... 15

Stability of Viral Pathogens in the Laboratory Environment
Hector N. Valtierra .................................................................................................... 21

Rapid and Biologically Safe Procedures for the Evaluation of Antigen-Specific T Cell Response to Microbial Pathogens That May Be Used in the BSL-3 and BSL-4 Environment
Chiara Agrati, Ilaria Volpi, Federico Martini, Cristina Gioia, Concetta Castilletti, Giuseppe Ippolito, Maria Rosaria Capobianchi, and Fabrizio Poccia ................................. 27

Development and Validation of a Pilot Scale Enhanced Biosafety Level Two Containment for Performance Evaluation of Produce Disinfection Technologies
Joseph E. Sites, Paul N. Walker, Angela Burke, and Bassam A. Annous ....................... 30

Evaluation of the Public Review Process and Risk Communication at High-Level Biocontainment Laboratories
Margaret S. Race ...................................................................................................... 45

(continued on page 2)
(continued from page 1)

Special Features

Biosafety Tips—Cell Sorters Present Containment Challenges
Karen B. Byers ............................................................................................................57

Molecular Biosafety—A Reassessment of Adeno-Associated Virus (AAV) Vector Risks
That Takes New Information on Insertional Mutagenesis into Account
Margy S. Lambert .............................................................................................................59

Capsule—Effectiveness of Personal Protective Measures to Prevent Lyme Disease
Ed Krisiunas ....................................................................................................................63

Ask the Experts—Biosafety Cabinets, Containment Facility Maintenance, and More
John H. Keene ................................................................................................................64

ABSA News

Tradeline Publications: Battelle Increases BSL-3 and ABSL-3 Research Space .......................66

2007 ABSA Service Award Recipients ..................................................................................69

2007 ABSA Conference Sponsors ..........................................................................................70

New ABSA Members for 2008 ..............................................................................................72

Calendar of Events ..............................................................................................................77

About the Cover

United States Department of Agriculture, Eastern Regional Research Center, Wyndmoor, Pennsylvania, personnel
are inspecting cantaloupes during a wash treatment at the Biosafety Level-2 pilot scale, Produce Processing Facility. The
processing (dump) tank in the foreground is a custom fabricated jacketed 1,000 liter vessel constructed of 304L stainless
steel, and built to Dairy Industry Standard 3A for stainless steel sanitary finish. Dump tank operation is fully automated
through a data acquisition and control system developed at the USDA using National Instruments components and
software. This control system allows researchers at the USDA to adjust the temperature between 3°C and 96°C and the
residence time between 30 seconds and 10 minutes during produce wash treatment studies. Such studies are designed to
determine the efficacy of conventional and experimental wash treatments on inactivating spoilage and human pathogens
on produce surfaces. To learn more about the influence of biosafety on food safety and research read the article on
pages 30-44 by Joseph E. Sites, Paul N. Walker, Angela Burke, and Bassam A. Annous. (Photo courtesy of Paul Pierlott.)
Authorization to Copy: No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, electrostatic, magnetic tape, photocopying, recording, or otherwise, without permission in writing from the copyright holder. The copyright restrictions of Title 17 of the United States Code do not apply to the articles prepared by employees of the U.S. Federal government.

Change of Address: A change of address notice should be sent at least 6 weeks in advance to the ABSA Office to ensure that all mailings, including the journal and newsletter, will reach you. ABSA is not responsible for misrouted mail as a result of insufficient notification of an address change. Undelivered copies resulting from an insufficient address change notification will not be replaced, but issues may be purchased at the single issue price as detailed above.

ABSA Office
American Biological Safety Association
1200 Allanson Road, Mundelein, IL 60060-3808, USA
1-866-425-1385 (toll free) / 847-949-1517 / Fax 847-566-4580
E-mail: absa@absa.org / Web Site: www.absa.org

Advertising Rates

<table>
<thead>
<tr>
<th>Rates</th>
<th>1x</th>
<th>2x</th>
<th>4x</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside back cover</td>
<td>$1,600</td>
<td>$1,440</td>
<td>$1,360</td>
</tr>
<tr>
<td>Inside front cover</td>
<td>$1,200</td>
<td>$1,080</td>
<td>$1,020</td>
</tr>
<tr>
<td>Inside back cover</td>
<td>$1,200</td>
<td>$1,080</td>
<td>$1,020</td>
</tr>
<tr>
<td>Full page</td>
<td>$1,000</td>
<td>$900</td>
<td>$850</td>
</tr>
<tr>
<td>1/2 page</td>
<td>$600</td>
<td>$540</td>
<td>$510</td>
</tr>
<tr>
<td>1/4 page</td>
<td>$400</td>
<td>$360</td>
<td>$340</td>
</tr>
</tbody>
</table>

Color rates: $350 for first color (after black) and $300 each additional color. 15% discount for agencies (orders must be supplied on agency letterhead).

Mechanical Requirements

<table>
<thead>
<tr>
<th></th>
<th>Width</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside back cover (full bleed)</td>
<td>8-1/2&quot;</td>
<td>11&quot;</td>
</tr>
<tr>
<td>Inside front cover (full bleed)</td>
<td>8-1/2&quot;</td>
<td>11&quot;</td>
</tr>
<tr>
<td>Inside back cover (full bleed)</td>
<td>8-1/2&quot;</td>
<td>11&quot;</td>
</tr>
<tr>
<td>Full page</td>
<td>8-1/2&quot;</td>
<td>11&quot;</td>
</tr>
<tr>
<td>1/2 page - horizontal</td>
<td>7&quot;</td>
<td>4-7/8&quot;</td>
</tr>
<tr>
<td>1/2 page - vertical</td>
<td>3-3/8&quot;</td>
<td>10&quot;</td>
</tr>
<tr>
<td>1/4 page</td>
<td>3-7/8&quot;</td>
<td>4-7/8&quot;</td>
</tr>
</tbody>
</table>

Trim size—8-1/2” x 11”

Film—133 line screen, right reading, emulsion side down, color separated

Submission Deadlines

<table>
<thead>
<tr>
<th></th>
<th>April 20 for Summer issue</th>
<th>October 15 for Winter issue</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 20 for Spring issue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 20 for Fall issue</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The publication of any advertisement by this journal is not an endorsement of the advertiser or of the products or services advertised. ABSA is not responsible for any claims made in any advertisement.
**Vision**

ABSA, the leader in the profession of biological safety.

**Mission Statement**

The American Biological Safety Association is dedicated to expanding biological safety awareness to prevent adverse occupational and environmental impact from biohazards.

**Goals**

- Expand professional and public awareness of biological safety through effective communication.
- Participate in the development of biological safety and biosecurity standards, guidelines, and regulations.
- Develop ABSA as the recognized resource for professional and scientific expertise in biological safety and biosecurity.
- Advance biological safety as a scientific discipline through education, research, and professional development.

**Officers**

**President**
Christina Z. Thompson, Thompson Biosafety LLC

**President-Elect**
Robert P. Ellis, Colorado State University

**Secretary**
Paul J. Meechan, Merck Research Laboratories

**Treasurer**
Leslie Delpin, University of Connecticut

**Past-President**
Robert J. Hawley, Midwest Research Institute

**Council Members**
LouAnn Burnett, Vanderbilt University
Joseph Kanabrocki, Washington University–St. Louis
Joseph P. Kozlovac, USDA Agricultural Research Service
Janet Peterson, University of Maryland

**Executive Director**
Edward John Stygar, III

**Affiliates and Affiliated Biosafety Organizations**

- ABSA CANADA: www.absa-canada.org
- ANBio: www.anbio.org.br
- BIONET: www.socalbionet.org
- Biological Safety Information Network: biosafety@comcast.net
- New England Biological Safety Association: www.duke.edu/~alder002
- CHABSA: www.chabsa.org
- EBASA: www.ebsaweb.eu
- MA BSP: www.malsa.org
- SEBSA: www.sebsa.org
- Taiwanese Biological Safety Association (TBSA): karen_byers@dfci.harvard.edu
- Japanese Biological Safety Association (JBSA): (Affiliate status is in process.)
- MABION: www.mabion.org
- NBSA: agwoodar@aol.com
- A-PBA: ling.ai.ee@sgh.com.sg
- Taiwanese Biological Safety Association (TBSA)
- Japanese Biological Safety Association (JBSA): huanglee@ms23.hinet.net
- k sugi@nih.go.jp
In November 2007, I had the pleasure to be one of ABSA's representatives to the final CEN workshop to develop an international laboratory standard for management of biological risks. CEN is the European Committee for Standardization. Although the CEN process was used, this is an international stakeholders' agreement, not a European standard. Adherence is completely voluntary. The workshop was held in Brussels, with about 60 participants in attendance, and led by Drs. Stefan Wagener and Gary Burns. Countries represented included the United States, Canada, Argentina, Australia, United Kingdom, Ireland, Belgium, Germany, Netherlands, Norway, Spain, Sweden, Switzerland, Hungary, Kazakhstan, China, Japan, and Singapore. Participants represented academia, government, and private industry.

The purpose of this third workshop was to review and come to agreement on changes to the standards document that had been developed over the course of the two previous workshops and public comment period. As was required by CEN, the entire process had to be completed in one year. The participants carefully reviewed and discussed the 102 page document and 800 comments over a two-day period. The document was revised using a consensus process and accepted by all participants.

It will now be a CEN Workshop Agreement titled Laboratory Biosafety Management Standard, and will be in effect for three years. At the end of that time, the participants will reconvene and decide on the future of the Agreement. One option for consideration is a future ISO standard. The next efforts (Phase II) will center on establishing an accreditation and certification system, which will include standards and qualifications for certifiers as well as certification protocols. This will not be a trivial task, but the stakeholders are determined to see it succeed.

We as ABSA members truly can be proud of this effort and the commitment and dedication by our past Council to see this finalized. In particular, my personal thanks go to Betsy Gilman Duane, Glenn Funk, Robert Hawley, and Patty Olinger for assisting and contributing to this great effort.

Earlier this month, ABSA received IACET accreditation, which means we can now offer Continuing Education Units (CEUs) for our courses. IACET is the internationally recognized organization for standards and authorization for continuing education and training. The CEU was created to: 1) provide a standard unit of measure; 2) quantify continuing adult education and training activities; and 3) serve the diversity of providers, activities, and purposes in adult education.

The ABSA Office submitted our application last spring. An IACET representative conducted a site visit at the office in early January, which LouAnn Burnett and Michelle McKinney attended. The site visit went very well, and ABSA was granted accreditation. This means you will receive CEUs for all ABSA courses you complete, which will aid all holders of the National Registry of Microbiologists (NRM) and other certifications in meeting their needs for recertification. Our thanks go to the ABSA Office staff, LouAnn, and Michelle for all their hard work and for representing our organization and educational offerings so well!

The Elizabeth R. Griffin Research Foundation, Inc. has generously provided funds to ABSA over the past few years. The Griffin Foundation is a nonprofit organization that promotes safe research practices and helps fund further research in the areas of zoonotic diseases. The funds are to be used for projects or activities which align with the goals of both organizations. Previous allocations of Griffin Foundation funds have been used to produce and distribute the Animal Biosafety DVD.

In 2008, ABSA has $20,000 of funding for activities which further our mutual goals. The precise type of activity(ies) is not prescribed by either organization; rather, we welcome a variety of requests. A Request for Proposal (RFP) form is on the ABSA web site. We know we have creative and knowledgeable members and strongly encourage everyone with ideas to apply for use of the Griffin Foundation funds. If you have an idea, please submit it!
Comparative Analysis of the Fourth and Fifth Editions of Biosafety in Microbiological and Biomedical Laboratories Section IV (BSL2-4)

Charles J. Crews and Edward E. Gaunt
Constella Group, LLC, Atlanta, Georgia

Abstract

We developed a matrix of changes between Section IV of the current and former editions of Biosafety in Microbiological and Biomedical Laboratories (BMBL), focusing on biosafety levels two, three, and four (BSL2, BSL3, and BSL4). Citations containing multiple statements were subdivided into individually addressable statements and statements with similar/identical scope were aligned, allowing for a precise comparative analysis. In addition, statements were categorized for further analysis based on the subject of the change, the type of change, and the outcome of the change. Although the Fifth Edition of BMBL (BMBL5) proved to be a close facsimile of its predecessor, we identified 375 total changes, including 165 novel statements not addressed in the former edition. Over 65% (110) of these changes were found in statements pertaining to BSL4 containment. In our study, we further examine these edition differences and identify key areas where those entities responsible for compliance with these new recommendations may wish to focus their efforts.

Introduction

First introduced in 1984, BMBL is an advisory document recommending best practices for the safe conduct of work in biomedical and clinical laboratories. Since its inception, it has become one of the most frequently used codes of practice in biosafety, and an authoritative reference for: the development of laboratory policies and procedures, the construction of new laboratories, and the renovation of existing laboratories (U.S. Department of Health and Human Services, 1999). Over the past two decades, periodic updates have been made to the BMBL to “refine guidance based on new knowledge and experiences and to address contemporary issues that present new risks that confront laboratory workers and the public health” (U.S. Department of Health and Human Services, 2007). In February 2007, a consortium of individuals from the Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) released the fifth edition of the BMBL, which contained a number of revisions and additions from the former, including:

- Added guidance on laboratory biosecurity and risk assessment.
- Added guidance on agricultural Biosafety Level 3 (BSL3-Ag) laboratories.
- Revisions and additions to agent summary statements.
- Expanded guidance on a number of topics, including decontamination, sterilization, occupational medicine, and immunization.

We report here the development of a change matrix identifying content changes to Section IV (Laboratory Biosafety Level Criteria) between the Fourth Edition of BMBL (BMBL4) and BMBL5, focusing specifically on BSL2-4.

Methods

The Laboratory Biosafety Level Criteria sections from BMBL4 and BMBL5 were analyzed and a matrix of changes was developed for BSL2-BSL4. The PDF version of BMBL5, released in November 2007 and available at www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf, was used for this analysis. Both sections were transferred electronically to a Microsoft Excel spreadsheet and separated by citation. Citations containing multiple statements were further subdivided into individually addressable statements to facilitate a detailed comparative analysis independent of simple structural and format differences not affecting meaning. BMBL4 statements were then reorganized and horizontally aligned with BMBL5 statements having a similar or identical scope. The original BMBL4 citations were preserved for cross-referencing. Each statement, or statement pair, was then systematically categorized based on subject, change type, and outcome, for further analysis.

Based upon observed trends in BMBL topics and laboratory commonalities, the following 17 biosafety-related subjects were determined and then assigned to each statement, or statement pair:

- Access
- Biological Safety Cabinet (BSC) design
- BSC exhaust
- BSC usage
- Decontamination
• Doors/Windows
• Exposures (lab-related accidents/illnesses)
• Immunization/Serum Collection
• Laboratory construction
• Laboratory furnishings (seating, furniture, carpeting)
• Laboratory ventilation (filtration, vacuums)
• Miscellaneous (food, cosmetics, pest control)
• Personal Protective Equipment (PPE)
• Sharps (glassware, needles, syringes)
• Signage
• Sinks/Eyewashes/Fountains
• Techniques (pipetting, animal use, storage, transfer)
• Training/Policies/Standardized Operating Procedures

Six change types, defined below, were then assigned to the data:
• No change—the statement scopes are identical; any rewording or reorganization does not affect meaning or potential interpretation.
• Not currently addressed—the BMBL4 statement is not addressed in BMBL5.
• Not previously addressed—the BMBL5 statement was not addressed in BMBL4.
• Rewording—the statement scopes are similar; however, the change affects meaning or potential interpretation.
• Scope expansion—the statement scopes are similar; however, the BMBL5 statement has been further expanded to include additional details.
• Scope generalization—the statement scopes are similar; however, the BMBL5 statement has been further generalized to be more all-encompassing.

Finally, three change outcomes were assigned to the data: those requiring only a change in documentation (such as recommendations for the establishment of written manuals), those requiring a change in practice (such as guidance on infectious waste decontamination procedures), and those requiring a facility change (such as guidance on laboratory ventilation requirements). The change matrix tool uses a basic Microsoft Excel filter function to allow users to sort the data based on the aforementioned categorizations as well as by BSL. We are providing the change matrix tool electronically to interested individuals free of charge upon request. In addition, a brief summary of major changes is presented herein.

Results

An excerpt of the change matrix covering the BSL2 subsection only is shown in Figure 1. For a complete change matrix covering BSL2-4, please contact the author via e-mail at ccrews@constellagroup.com or visit the ABSA web site at www.absa.org/word/BMBLChangeMatrix.xls where the change matrix tool has been posted. This file allows the user to filter and sort the various BMBL statements based on the criteria relevant to a particular laboratory situation. Five hundred twelve total statement/statement pairs were identified following the subdivision of citations and realignment of BMBL4 statements with similar/identical scope. Of this total, 375 unique changes were identified. Fifty-six percent (211) of these changes were identified as BSL4 statements whereas 26% (97) were identified as BSL3 changes and 18% (67) were identified as BSL2 changes. These results are summarized in Figure 2.

Forty-five percent (166) of the total changes were identified as being unique to BMBL5 (not addressed in the previous edition); 66% of the changes unique to BMBL5 (110) were identified as BSL4 statements; 15% (56) of the total changes were identified as being unique to BMBL4 (not addressed in the current edition); 16% (61) of the total changes were identified as scope generalizations, and 13% (50) were identified as scope expansions. Twelve percent (44) of the total changes were identified as rewordings affecting the statement’s meaning. Figure 3 is a summary of the percent of total changes by type.

Figure 4 summarizes the number of changes by subject. The three most frequent subjects for change are decontamination (62, 17%), laboratory ventilation (47, 13%), and personal protective equipment (PPE) (58, 16%). Other notable areas of change include biological safety cabinet (BSC) exhaust (26, 7%), laboratory construction/layout (35, 9%), sharps (25, 7%), and training/standard operating procedures (25, 7%).

Forty-seven percent (177) of the total number of changes between the two BMBL editions are practice changes, whereas 37% (140) are facility changes and 16% (58) are documentation changes. Seventy-one percent (99) of facility changes and 54% (95) of practice changes were identified at BSL4 changes. These data are summarized in Figure 5.

Figure 6 shows a concise summary of the major changes between the fourth and fifth editions of BMBL Section IV. The biosafety levels pertinent to each change, as well as to all applicable BMBL citations are documented. The summary focuses on major practice and facility changes that we have subjectively determined to have a high impact based upon a number of criteria, including: the immediacy of the safety concern, the level of resources required to comply with the change, and the severity of the consequences of non-compliance. The summary is intended to be a quick resource to assist biosafety professionals in concentrating their efforts on specific areas, and is not intended to be definitive or official reference material.

Discussion

Although the BMBL is not intended as a regulatory document, in some circumstances, compliance with the BMBL has been legally mandated. For biosafety professionals tasked with ensuring facility compliance with the BMBL, keeping abreast of changing guidance poses a
### Figure 1

A change matrix comparing 4th and 5th editions of *Biosafety in Microbiological and Biomedical Laboratories*, Section IV. This figure only presents BSL2 changes. For a complete change matrix covering BSL2-4, please contact the author via email at crewes@constellagroup.com or visit the ABSA web site at www.absa.org/word/MBMBLChangeMatrix.xls where the change matrix tool has been posted.

<table>
<thead>
<tr>
<th>Item #</th>
<th>BSL</th>
<th>Subject</th>
<th>BMBL-4 Section</th>
<th>BMBL-4</th>
<th>BMBL-5</th>
<th>Change Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BSL2</td>
<td>Access</td>
<td>BMBL-4: A1</td>
<td>Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.</td>
<td>BMBL-4: A1</td>
<td>The laboratory supervisor must enforce the institutional policies that control access to the laboratories.</td>
<td>Rewording</td>
</tr>
<tr>
<td>2</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: A3</td>
<td>Person wearing gloves after they handle sharp materials, before removing gloves, and before leaving the laboratory.</td>
<td>BMBL-4: A2</td>
<td>Persons must wear gloves after working with potentially hazardous materials and before leaving the laboratory.</td>
<td>Rewording</td>
</tr>
<tr>
<td>3</td>
<td>BSL2</td>
<td>Misc.</td>
<td>BMBL-4: A3</td>
<td>Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas.</td>
<td>BMBL-4: A3</td>
<td>Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human consumption must not be permitted in laboratory areas.</td>
<td>Expansion</td>
</tr>
<tr>
<td>4</td>
<td>BSL2</td>
<td>Misc.</td>
<td>BMBL-4: A3</td>
<td>Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.</td>
<td>BMBL-4: A3</td>
<td>Food must be stored outside the laboratory area in cabinets or refrigerators designated and used for this purpose.</td>
<td>No Change</td>
</tr>
<tr>
<td>5</td>
<td>BSL2</td>
<td>Techniques</td>
<td>BMBL-4: A4</td>
<td>All principles are promulgated.</td>
<td>BMBL-4: A4</td>
<td>All principles are promulgated.</td>
<td>No Change</td>
</tr>
<tr>
<td>6</td>
<td>BSL2</td>
<td>Techniques</td>
<td>BMBL-4: A4</td>
<td>Mechanical/peddling devices are used.</td>
<td>BMBL-4: A4</td>
<td>Mechanical/peddling devices are used.</td>
<td>No Change</td>
</tr>
<tr>
<td>7</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Precautions for the safe handling of sharps are instituted.</td>
<td>BMBL-4: A5</td>
<td>Precautions for the safe handling of sharps are instituted.</td>
<td>Expansion</td>
</tr>
<tr>
<td>8</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, aspiration of fluids from laboratory animals and diaphragm bottles.</td>
<td>BMBL-4: A5</td>
<td>Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, aspiration of fluids from laboratory animals and diaphragm bottles.</td>
<td>No Change</td>
</tr>
<tr>
<td>9</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Needles and syringes are used, rather than other means such as needles, scalpels, pipettes, and broken glass are manipulated by hand before disposal.</td>
<td>BMBL-4: A5</td>
<td>Needles and syringes are used, rather than other means such as needles, scalpels, pipettes, and broken glass are manipulated by hand before disposal.</td>
<td>Expansion</td>
</tr>
<tr>
<td>10</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Disposal of sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.</td>
<td>BMBL-4: A5</td>
<td>Disposal of sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.</td>
<td>No Change</td>
</tr>
<tr>
<td>11</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Only needle-locking syringe or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.</td>
<td>BMBL-4: A5</td>
<td>Only needle-locking syringe or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.</td>
<td>No Change</td>
</tr>
<tr>
<td>12</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Careful management of needles and other sharps are of primary importance.</td>
<td>BMBL-4: A5</td>
<td>Careful management of needles and other sharps are of primary importance.</td>
<td>No Change</td>
</tr>
<tr>
<td>13</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.</td>
<td>BMBL-4: A5</td>
<td>Needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal.</td>
<td>Generalization</td>
</tr>
<tr>
<td>14</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.</td>
<td>BMBL-4: A5</td>
<td>Needles and syringes must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal.</td>
<td>No Change</td>
</tr>
<tr>
<td>15</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.</td>
<td>BMBL-4: A5</td>
<td>Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.</td>
<td>No Change</td>
</tr>
<tr>
<td>16</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Syringes which re-sharps the needle, needless systems, and other safety devices are used when appropriate.</td>
<td>BMBL-4: A5</td>
<td>Syringes which re-sharps the needle, needless systems, and other safety devices are used when appropriate.</td>
<td>No Change</td>
</tr>
<tr>
<td>17</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A5</td>
<td>Broken glass are not to be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.</td>
<td>BMBL-4: A5</td>
<td>Broken glass are not to be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps.</td>
<td>No Change</td>
</tr>
<tr>
<td>18</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A6</td>
<td>Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.</td>
<td>BMBL-4: A6</td>
<td>Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.</td>
<td>NCA</td>
</tr>
<tr>
<td>19</td>
<td>BSL2</td>
<td>Sharps</td>
<td>BMBL-4: A6</td>
<td>Practice are should be substituted for exposure where ever possible.</td>
<td>BMBL-4: A6</td>
<td>Practice are should be substituted for exposure where ever possible.</td>
<td>No Change</td>
</tr>
<tr>
<td>20</td>
<td>BSL2</td>
<td>Techniques</td>
<td>BMBL-4: A6</td>
<td>All procedures are performed carefully to minimize the creation of splashes or aerosols.</td>
<td>BMBL-4: A6</td>
<td>All procedures are performed carefully to minimize the creation of splashes or aerosols.</td>
<td>No Change</td>
</tr>
<tr>
<td>21</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: A7</td>
<td>Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of potentially infectious material.</td>
<td>BMBL-4: A7</td>
<td>Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of potentially infectious material.</td>
<td>No Change</td>
</tr>
<tr>
<td>22</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: A7</td>
<td>All cultures, stock, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving.</td>
<td>BMBL-4: A7</td>
<td>All cultures, stock, and other potentially infectious materials are decontaminated before disposal by an approved decontamination method such as autoclaving.</td>
<td>Rewording</td>
</tr>
<tr>
<td>23</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: A7</td>
<td>Depending on where the decontamination will be performed, the following methods should be used prior to transport:</td>
<td>BMBL-4: A7</td>
<td>Depending on where the decontamination will be performed, the following methods should be used prior to transport:</td>
<td>No Change</td>
</tr>
<tr>
<td>24</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: A7</td>
<td>Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory.</td>
<td>BMBL-4: A7</td>
<td>Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leak-proof container and closed for transport from the laboratory.</td>
<td>No Change</td>
</tr>
<tr>
<td>25</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: A7</td>
<td>Materials to be decontaminated off-site, and the facility are in accordance with applicable local, state, and federal regulations before removal from the facility.</td>
<td>BMBL-4: A7</td>
<td>Materials to be decontaminated off-site, and the facility are in accordance with applicable local, state, and federal regulations before removal from the facility.</td>
<td>No Change</td>
</tr>
</tbody>
</table>

NPA = Not Previously Addressed; NCA = Not Currently Addressed; DC = Documentation Change; FC = Facility Change; PC = Policy/Procedural Change; NC = No Change
<table>
<thead>
<tr>
<th>Item #</th>
<th>BSL</th>
<th>Subject</th>
<th>BMBL-4 Section</th>
<th>BMBL-4</th>
<th>BMBL-5 Section</th>
<th>BMBL-5 (PDF version)</th>
<th>Change Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>BSL2</td>
<td>Signage</td>
<td>BMBL4: B3</td>
<td>A biohazard sign must be posted on the entrance to the laboratory when infectious agents are in use.</td>
<td>BMBL5: A9</td>
<td>A sign incorporating the universal/biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present.</td>
<td>Rewording</td>
<td>FC</td>
</tr>
<tr>
<td>27</td>
<td>BSL2</td>
<td>Signage</td>
<td>BMBL4: B3</td>
<td>Appropriate information to be posted includes the agent(s) in use, the biohazard level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.</td>
<td>BMBL5: A9</td>
<td>Posted information must include the laboratory's biohazard level, the supervisor's name (or other responsible personnel), telephone number, and required procedures for entering and exiting the laboratory.</td>
<td>Generalization</td>
<td>DC</td>
</tr>
<tr>
<td>28</td>
<td>BSL2</td>
<td>Signage</td>
<td>BMBL4: B3</td>
<td>Appropriate information to be posted includes the agent(s) in use.</td>
<td>BMBL5: A9</td>
<td>Appropriate information should be posted in accordance with the institutional policy.</td>
<td>Rewording</td>
<td>FC</td>
</tr>
<tr>
<td>29</td>
<td>BSL2</td>
<td>Misc.</td>
<td>BMBL4: A9</td>
<td>An insect and rodent control program is in effect.</td>
<td></td>
<td>An effective integrated pest management program is required. See Appendix G.</td>
<td>Expansion</td>
<td>PC</td>
</tr>
<tr>
<td>30</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B7</td>
<td>The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures.</td>
<td>BMBL5: A11</td>
<td>The laboratory supervisor must ensure that laboratory personnel receive appropriate training regarding their duties, the necessary precautions to prevent exposures, and exposure evaluation procedures.</td>
<td>Rewording</td>
<td>PC</td>
</tr>
<tr>
<td>31</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B7</td>
<td>Personnel must receive annual updates or additional training as necessary for procedural or policy changes.</td>
<td>BMBL5: A11</td>
<td>Personal health status may impact an individual's susceptibility to infection, ability to receive immunizations or prophylactic interventions. Therefore, all laboratory personnel, and particularly women of childbearing age should be provided with information regarding immune competence and conditions that may predispose them to infection.</td>
<td>NPA</td>
<td>PC</td>
</tr>
<tr>
<td>32</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: A11</td>
<td>An insect and rodent control program is in effect.</td>
<td></td>
<td>An effective integrated pest management program is required. See Appendix G.</td>
<td>Expansion</td>
<td>PC</td>
</tr>
<tr>
<td>33</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B3</td>
<td>The laboratory director establishes policies and procedures whereby only persons who are advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.</td>
<td>BMBL5: A11</td>
<td>All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements.</td>
<td>Generalization</td>
<td>PC</td>
</tr>
<tr>
<td>34</td>
<td>BSL2</td>
<td>Access</td>
<td>BMBL4: B1</td>
<td>Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.</td>
<td>BMBL5: B1</td>
<td>All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements.</td>
<td>Generalization</td>
<td>PC</td>
</tr>
<tr>
<td>35</td>
<td>BSL2</td>
<td>Access</td>
<td>BMBL4: B2</td>
<td>The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.</td>
<td>BMBL5: B1</td>
<td>All persons entering the laboratory must be advised of the potential hazards and meet specific entry requirements.</td>
<td>Generalization</td>
<td>PC</td>
</tr>
<tr>
<td>36</td>
<td>BSL2</td>
<td>Immunization/ Serum Collection</td>
<td>BMBL4: B4</td>
<td>Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).</td>
<td>BMBL5: B2</td>
<td>Laboratory personnel must receive medical surveillance and offered appropriate immunizations for agents handled or potentially present in the laboratory.</td>
<td>Expansion</td>
<td>PC</td>
</tr>
<tr>
<td>37</td>
<td>BSL2</td>
<td>Immunization/ Serum Collection</td>
<td>BMBL4: B5</td>
<td>Properly appropriate, consilering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.</td>
<td>BMBL5: B3</td>
<td>Such institution established policies and procedures describing the collection and storage of serum samples from at-risk personnel.</td>
<td>Generalization</td>
<td>DC</td>
</tr>
<tr>
<td>38</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B6</td>
<td>Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director.</td>
<td>BMBL5: B4</td>
<td>A laboratory-specific biosafety manual must be prepared and adopted as policy.</td>
<td>Generalization</td>
<td>DC</td>
</tr>
<tr>
<td>39</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B6</td>
<td>Personal are advised of special hazards and are required to read and follow instructions on practices and procedures.</td>
<td></td>
<td>Personal are advised of special hazards and are required to read and follow instructions on practices and procedures.</td>
<td>NCA</td>
<td>DC</td>
</tr>
<tr>
<td>40</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B4</td>
<td>The biosafety manual must be available and accessible.</td>
<td>BMBL5: B4</td>
<td>The biosafety manual must be available and accessible.</td>
<td>NPA</td>
<td>PC</td>
</tr>
<tr>
<td>41</td>
<td>BSL2</td>
<td>Training/SOPs</td>
<td>BMBL4: B5</td>
<td>The biosafety manual must be available and accessible.</td>
<td>BMBL5: B5</td>
<td>The biosafety manual must be available and accessible.</td>
<td>NPA</td>
<td>PC</td>
</tr>
<tr>
<td>42</td>
<td>BSL2</td>
<td>Techniques</td>
<td>BMBL4: B9</td>
<td>Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.</td>
<td>BMBL5: B6</td>
<td>Potentially infectious materials must be placed in a durable, leak proof container during collection, handling, processing, storage, or transport.</td>
<td>Generalization</td>
<td>PC</td>
</tr>
<tr>
<td>43</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL4: B10</td>
<td>Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials.</td>
<td>BMBL5: B7</td>
<td>Laboratory equipment should be routinely decontaminated, as well as, after spills, splashes, or other potential contamination.</td>
<td>Generalization</td>
<td>PC</td>
</tr>
</tbody>
</table>

NPA = Not Previously Addressed; NCA = Not Currently Addressed; DC = Documentation Change; FC = Facility Change; PC = Policy/Procedural Change; NC = No Change
### C. Safety Equipment (Primary Barriers and Personal Protective Equipment)

<table>
<thead>
<tr>
<th>Item #</th>
<th>BSL</th>
<th>Subject</th>
<th>BMBL-4 Section</th>
<th>BMBL-4</th>
<th>BMBL-5 Section</th>
<th>BMBL-5 (PDF version)</th>
<th>Change Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>44</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: B10</td>
<td>BMBL5: B7-b</td>
<td>Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.</td>
<td>NPA PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL-4: B10</td>
<td>BMBL5: B7-b</td>
<td>Equipment must be decontaminated before repair, maintenance, or removal from the laboratory.</td>
<td>Generalization PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>BSL2</td>
<td>Exposures</td>
<td>BMBL-4: B11</td>
<td>BMBL5: B8</td>
<td>Details of exposure to infectious materials are immediately reported to the laboratory supervisor.</td>
<td>NPA PC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>BSL2</td>
<td>Exposures</td>
<td>BMBL-4: B11</td>
<td>BMBL5: B8</td>
<td>Details of exposure to infectious materials are immediately reported to the laboratory supervisor.</td>
<td>No Change NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>BSL2</td>
<td>Exposures</td>
<td>BMBL-4: B11</td>
<td>BMBL5: B8</td>
<td>Animals not involved in the work being performed are not permitted in the laboratory.</td>
<td>No Change NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>BSL2</td>
<td>Techniques</td>
<td>BMBL-4: B12</td>
<td>BMBL5: B9</td>
<td>Animals and plants not associated with the work being performed must not be permitted in the laboratory.</td>
<td>No Change NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>BSL2</td>
<td>Techniques</td>
<td>BMBL-4: B10</td>
<td>BMBL5: B10</td>
<td>All procedures involving the manipulation of infectious material that may generate an aerosol should be conducted within a BSC or other physical containment devices.</td>
<td>NPA PC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### BMBL-4
- **C1**: High concentrations or large volumes of infectious agents are used. Such materials may require centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonated eggs.

#### BMBL-5
- **C1-b**: High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory using sealed rotor heads or centrifuge safety cups.
- **C2**: Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.
- **C3**: Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.
- **C4**: Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory.

#### BMBL-5 (PDF version)
- **C1**: High concentrations or large volumes of infectious agents are used. Such materials may require centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonated eggs.

**NPA** = Not Previously Addressed; **NCA** = Not Currently Addressed; **DC** = Documentation Change; **FC** = Facility Change; **PC** = Policy/Procedural Change; **NC** = No Change
## Figure 1 (Con’t.)

<table>
<thead>
<tr>
<th>Item #</th>
<th>BSL</th>
<th>Subject</th>
<th>BMBL-4 Section</th>
<th>BMBL-5 Section</th>
<th>Change Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>BSL2</td>
<td>PPE</td>
<td>BMBL4: C4</td>
<td>BMBL5: C4-c</td>
<td>Expansion</td>
<td>FC</td>
</tr>
<tr>
<td>69</td>
<td>BSL2</td>
<td>PPE</td>
<td>BMBL4: C4</td>
<td>BMBL5: C4-c</td>
<td>Rewording</td>
<td>PC</td>
</tr>
<tr>
<td>70</td>
<td>BSL2</td>
<td>PPE</td>
<td>NPA DC</td>
<td>NPA FC</td>
<td></td>
<td>NCA DC</td>
</tr>
<tr>
<td>71</td>
<td>BSL2</td>
<td>PPE</td>
<td>BMBL4: C4</td>
<td>BMBL5: C4-c</td>
<td>Generalization</td>
<td>PC</td>
</tr>
<tr>
<td>72</td>
<td>BSL2</td>
<td>PPE</td>
<td>BMBL5: C5</td>
<td></td>
<td></td>
<td>NPA PC</td>
</tr>
</tbody>
</table>

### D. Laboratory Facilities (Secondary Barriers)

#### Lab Construction

<table>
<thead>
<tr>
<th>Item #</th>
<th>BSL</th>
<th>Subject</th>
<th>BMBL-4 Section</th>
<th>BMBL-5 Section</th>
<th>Change Type</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td>BSL2</td>
<td>Doors/Windows</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Expansion</td>
<td>FC</td>
</tr>
<tr>
<td>74</td>
<td>BSL2</td>
<td>Lab Construction</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NCA DC</td>
</tr>
<tr>
<td>75</td>
<td>BSL2</td>
<td>Sinks/Eyewash</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>76</td>
<td>BSL2</td>
<td>Sinks/Eyewash</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>77</td>
<td>BSL2</td>
<td>Sinks/Eyewash</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>78</td>
<td>BSL2</td>
<td>Lab Construction</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>79</td>
<td>BSL2</td>
<td>Lab Furnishings</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>80</td>
<td>BSL2</td>
<td>Lab Furnishings</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>81</td>
<td>BSL2</td>
<td>Lab Construction</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>82</td>
<td>BSL2</td>
<td>Lab Construction</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>83</td>
<td>BSL2</td>
<td>Lab Furnishings</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>84</td>
<td>BSL2</td>
<td>Doors/Windows</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>85</td>
<td>BSL2</td>
<td>Doors/Windows</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>86</td>
<td>BSL2</td>
<td>BSC Exhaust</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>87</td>
<td>BSL2</td>
<td>BSC Exhaust</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>88</td>
<td>BSL2</td>
<td>Lab Ventilation</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>89</td>
<td>BSL2</td>
<td>Lab Ventilation</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>90</td>
<td>BSL2</td>
<td>Lab Ventilation</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>91</td>
<td>BSL2</td>
<td>Sinks/Eyewash</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>92</td>
<td>BSL2</td>
<td>Lab Construction</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NCA DC</td>
</tr>
<tr>
<td>93</td>
<td>BSL2</td>
<td>Lab Ventilation</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>94</td>
<td>BSL2</td>
<td>BSC Exhaust</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>95</td>
<td>BSL2</td>
<td>BSC Exhaust</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA FC</td>
</tr>
<tr>
<td>96</td>
<td>BSL2</td>
<td>BSC Exhaust</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA PC</td>
</tr>
<tr>
<td>97</td>
<td>BSL2</td>
<td>Decon.</td>
<td>BMBL4: D1</td>
<td>BMBL5: D1</td>
<td>Description</td>
<td>NPA DC</td>
</tr>
</tbody>
</table>

NPA = Not Previously Addressed; NCA = Not Currently Addressed; DC = Documentation Change; FC = Facility Change; PC = Policy/Procedural Change; NC = No Change
**Figure 2**
A graphical representation of the percent of total BMBL Section IV changes by Biosafety Level.

![BSL Level Percentages](image1)

**Figure 3**
A graphical representation of the percent of total BMBL Section IV changes by type (n = 375).

![Type Percentages](image2)

**Figure 4**
A comparison of the number of BMBL Section IV changes by subject. White bars indicate the three most common subjects for change.

![Subject Changes](image3)

**Figure 5**
A graphical representation of the number of document, practice, and facility changes between the 4th and 5th editions of BMBL by biosafety level.

![BSL Changes](image4)
## Figure 6
A summary of major changes between the 4th and 5th editions of BMBL Section IV, BSL2-BSL4.

<table>
<thead>
<tr>
<th>Biosafety Level</th>
<th>Major Changes</th>
<th>Applicable BMBL Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSL2, BSL3, BSL4</td>
<td>The restriction of access to personnel who are at risk of acquiring infection is not addressed in BMBL5. BMBL5 states that all lab personnel should be provided with information regarding “immune competence and conditions that may predispose them to infection.” Lab personnel are also encouraged to self-identify.</td>
<td>BMBL4: BSL2 B1; BSL3 B2; BSL4 B1. BMBL5: BSL2 A11; BSL3 A11; BSL4 A11.</td>
</tr>
<tr>
<td>BSL2, BSL3</td>
<td>“Laboratory personnel must be provided with medical surveillance.”</td>
<td>BMBL5: BSL2 B2; BSL3 B3.</td>
</tr>
<tr>
<td>BSL2, BSL3, BSL4</td>
<td>“Incidents that may result in exposure to infectious materials must be immediately evaluated and treated according to procedures described in the laboratory biosafety safety manual.”</td>
<td>BMBL5: BSL2 B8; BSL3 B8; BSL4 B8.</td>
</tr>
<tr>
<td>BSL2</td>
<td>“All procedures involving the manipulation of infectious materials that may generate an aerosol should be conducted within a BSC or other physical containment devices.”</td>
<td>BMBL5: BSL2 B10</td>
</tr>
<tr>
<td>BSL2, BSL3, BSL4</td>
<td>Enhanced guidance on eye and face protection use, decontamination and disposal (See Citations).</td>
<td>BMBL5: BSL2 C3; BSL3 C3; BSL4 C(A)3</td>
</tr>
<tr>
<td>BSL2, BSL3, BSL4</td>
<td>Enhanced guidance on glove selection, use, and disposal (See Citations).</td>
<td>BMBL5: BSL2 C4; BSL3 C4; BSL4 C(A)4, C(B)3, C(B)4</td>
</tr>
<tr>
<td>BSL2</td>
<td>“Vacuum lines should be protected with High Efficiency Particulate Air (HEPA) filters, or their equivalent. Filters must be replaced as needed. Liquid disinfectant traps may be required.”</td>
<td>BMBL5: BSL2 D7</td>
</tr>
<tr>
<td>BSL2, BSL3, BSL4</td>
<td>Enhanced guidance on Class II BSC exhaust (See Citations).</td>
<td>BMBL5: BSL2 D10; BSL3 D10; BMBL4: C(B)1, D(A)10, D(B)10.</td>
</tr>
<tr>
<td>BSL3</td>
<td>“Laboratory doors must be self closing and have locks in accordance with the institutional policies.”</td>
<td>BMBL5: BSL3 D1</td>
</tr>
<tr>
<td>BSL3</td>
<td>“If the laboratory is segregated into different laboratories, a sink must also be available for hand washing in each zone.”</td>
<td>BMBL5: BSL3 D2</td>
</tr>
<tr>
<td>BSL3</td>
<td>Enhanced guidance on HEPA filtration of laboratory exhaust air (See Citation).</td>
<td>BMBL5: BSL3 D14</td>
</tr>
<tr>
<td>BSL4</td>
<td>“Mechanical pipetting devices must be used.”</td>
<td>BMBL5: BSL4 A4</td>
</tr>
<tr>
<td>BSL4</td>
<td>“The interior of the Class III cabinet as well as all contaminated plenums, fans and filters must be decontaminated using a validated gaseous or vapor method.”</td>
<td>BMBL5: BSL4 B7-b</td>
</tr>
<tr>
<td>BSL4</td>
<td>Enhanced guidance on autoclave design and operation (See Citation).</td>
<td>BMBL5: BSL4 B10</td>
</tr>
<tr>
<td>BSL4 (cabinet lab)</td>
<td>Enhanced guidance on Class III BSC design, exhaust, and usage in the cabinet laboratory (See Citation).</td>
<td>BMBL5: BSL4 C(A)1.</td>
</tr>
<tr>
<td>BSL4 (cabinet lab)</td>
<td>Enhanced guidance on personal and protective clothing usage and decontamination in the cabinet laboratory (See Citation).</td>
<td>BMBL5: BSL4 C(A)2.</td>
</tr>
<tr>
<td>BSL4 (cabinet lab); BSL4 (suit lab)</td>
<td>“An automatically activated emergency power source must be provided at a minimum for the laboratory exhaust system, life support systems, alarms, lighting, entry and exit controls, BSCs, and door gaskets. Monitoring and control systems for air supply, exhaust, life support, alarms, entry and exit, and security systems should be on an uninterrupted power supply (UPS).”</td>
<td>BMBL5: BSL4 D(A)1, D(B)1.</td>
</tr>
<tr>
<td>BSL4 (cabinet lab)</td>
<td>“All sinks in the room(s) containing the Class III BSC and the inner (dirty) change room must be connected to the wastewater decontamination system.”</td>
<td>BMBL5: BSL4 D(A)2.</td>
</tr>
<tr>
<td>BSL4 (cabinet lab); BSL4 (suit lab)</td>
<td>Enhanced guidance on liquid and gas services (See Citations).</td>
<td>BMBL5: BSL4 D(A)3, D(B)3.</td>
</tr>
</tbody>
</table>
**Figure 6 (Con’t.)**

<table>
<thead>
<tr>
<th>Biosafety Level</th>
<th>Major Changes</th>
<th>Applicable BMBL Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSL4 (cabinet lab)</td>
<td>“Redundant supply fans are recommended. Redundant exhaust fans are required.”</td>
<td>BMBL5: BSL4 D(A)9</td>
</tr>
<tr>
<td>BSL4 (cabinet lab)</td>
<td>“The design of the HEPA filter housing must have gas-tight isolation dampers; decontamination ports; and ability to scan each filter assembly for leaks.”</td>
<td>BMBL5: BSL4 D(A)9</td>
</tr>
<tr>
<td>BSL4 (cabinet lab)</td>
<td>“Class III BSCs must be directly and independently exhausted through two HEPA filters in series.”</td>
<td>BMBL5: BSL4 D(A)9</td>
</tr>
<tr>
<td>BSL4 (cabinet lab); BSL4 (suit lab)</td>
<td>“Access to the exit side of the pass-through shall be limited to those individuals authorized to be in the BSL-4 laboratory.”</td>
<td>BMBL5: BSL4 D(A)11, D(B)11.</td>
</tr>
<tr>
<td>BSL4 (cabinet lab); BSL4 (suit lab)</td>
<td>“Gas and liquid discharge from the autoclave chamber must be decontaminated. When feasible, autoclave decontamination processes should be designed so that over-pressurization cannot release unfiltered air or steam exposed to infectious material to the environment.”</td>
<td>BMBL5: BSL4 D(A)13; D(B)13</td>
</tr>
<tr>
<td>BSL4 (cabinet lab); BSL4 (suit lab)</td>
<td>“Provisions for emergency communication and access/egress must be considered.”</td>
<td>BMBL5: BSL4 D(A)15, D(B)15.</td>
</tr>
<tr>
<td>BSL4 (suit lab)</td>
<td>“In the event of an emergency exit or failure of chemical shower system a method for decontaminating positive pressure suits, such as a gravity fed supply of chemical disinfectant, is needed.”</td>
<td>BMBL5: BSL4 D(B)1</td>
</tr>
<tr>
<td>BSL4 (suit lab)</td>
<td>“Only laboratories with the same HVAC requirements (i.e., other BSL-4 labs, ABSL-4, BSL-3 Ag labs) may share ventilation systems if each individual laboratory system is isolated by gas tight dampers and HEPA filters.”</td>
<td>BMBL5: BSL4 D(A)9, D(B)9.</td>
</tr>
<tr>
<td>BSL4 (suit lab)</td>
<td>“Autoclaves that open outside of the laboratory must be sealed to the primary wall.”</td>
<td>BMBL5: BSL4 D(B)13</td>
</tr>
</tbody>
</table>

**Figure 7**

A flow chart representing the utility of the BMBL change matrix methodology for future BMBL and other regulatory and/or guidance document updates.
unique challenge since the revision summaries of rapidly and dramatically changing guidance documents are often scant or non-existent. The change matrix we have developed is intended to be a resource for biological safety officers, investigators, and others with a vested interest in biosafety to assist in the identification of the issues most pertinent to their facility or institution. Individual statements or statement pairs have been presorted based on a number of criteria therefore allowing the individual user the freedom to filter the data based on their specific needs. For instance, an individual primarily interested in facility changes affecting laboratory exhaust requirements may sort through the changes based on those specific criteria.

The change matrix we have created presents a systematic and streamlined methodology for identifying changes in the Laboratory Biosafety Level Criteria sections between the fourth and fifth editions of BMBL. An overview of this process is represented graphically in Figure 7. We believe this methodology can also be applied to future revisions as well as to other elaborate guidance documents where a precise awareness of changing guidance is crucial to safety and regulatory compliance. In addition, the organization of the matrix makes it possible for the individual user to add additional criteria (e.g., level of resource investment) and search for them against existing criteria.

Authors’ Note

The information contained herein does not necessarily represent the position of the federal government.

References


---

**Inactivation of* Francisella tularensis* Schu S4 in a Biological Safety Cabinet Using Hydrogen Peroxide Fumigation**

James V. Rogers and Young W. Choi

Battelle Memorial Institute, Columbus, Ohio

**Abstract**

This study evaluated the inactivation of Francisella tularensis Schu S4 on various materials (acrylic, glass, polyamide, polyethylene, polypropylene, silicone rubber, and stainless steel) using hydrogen peroxide fumigation of a Class III Biological Safety Cabinet (BSC III). A suspension of *F. tularensis* Schu S4 (7 x 10⁷ CFU) was dried on seven different types of test surfaces and exposed to vaporous hydrogen peroxide (VHP) fumigation for a contact time of two hours. Qualitative growth assessment showed that VHP exposure inactivated *F. tularensis* on all replicates of the seven test materials up to four days post-exposure. The effectiveness of VHP fumigation on the growth of biological indicators (*Bacillus subtilis* or *Geobacillus stearothermophilus*) and spore strips (*Bacillus atrophaeus*) was evaluated in parallel as a qualitative assessment of decontamination. At one and four days post-exposure, decontaminated biological indicators and spore strips exhibited no growth, while the non-decontaminated samples displayed growth. This study provides information for using VHP fumigation as an alternative approach for the decontamination of virulent *F. tularensis* when the current accepted method of 10% household bleach followed by 70% alcohol may not be practical for decontamination of a BSC III.

**Introduction**

The gram-negative cocccobacillus, *Francisella tularensis* (formerly known as *Pasteurella tularensis*), is the etiologic agent of the zoonotic disease tularemia. *Francisella tularensis* is classified as a Category A select agent due to its infectivity and capacity to cause illness and death, thus heightening the concern of using this microorganism as a potential biological weapon (Dennis et al., 2001; Peterson & Schriefer, 2005). Such concerns have prompted decades of research investigating and developing vaccines and medical countermeasures against *F. tularensis* infection (Ellis et al., 2002).
In the environment, various factors such as temperature, relative humidity, sunlight, mode of dispersion (e.g., aerosols), and environmental material matrices (soil, water, etc.) influence the duration that *F. tularensis* can remain viable, which can last for weeks or months (Anda et al., 2001; Feldman et al., 2001; Abd et al., 2003; Feldman, 2003). The persistence of *F. tularensis* necessitates effective decontamination technologies and applications since only a few viable organisms are sufficient to cause potential infection (Saslaw & Carlisle, 1969). This is especially crucial in laboratories conducting research under Biosafety Level 3 (BSL-3) containment where *F. tularensis* is potentially applied or dispersed in various forms such as liquids or aerosols. Although *F. tularensis* is susceptible to heat inactivation (Ehrlich & Miller, 1973; Anda et al., 2001), there are often situations in which potentially contaminated items such as surfaces and sensitive equipment cannot be treated by autoclaving, heat, or liquid decontaminants. Therefore, fumigating agents such as VHP are often more suitable for decontaminating these materials within equipment in a BSL-3 containment facility.

Currently, the decontamination approach for treating the intentional release of *F. tularensis* on contaminated inanimate surfaces is achieved by spraying the suspected contaminated area with a 10% household bleach solution (one part household bleach and nine parts water) for at least a 10-minute contact time, followed by further cleaning with a 70% alcohol solution (Dennis et al., 2001). As a fumigant, hydrogen peroxide has been shown to decontaminate a wide range of microorganisms; however, efficacy against *F. tularensis* has not been demonstrated. Therefore, this study was conducted to evaluate the decontamination of *F. tularensis* using VHP fumigation of a BSC III.

**Materials and Methods**

**Test Organisms**

The virulent strain of *F. tularensis* Schu S4 was propagated on Chocolate II agar with hemoglobin and IsoVitaleX™ (CII+H+1 agar; BD Diagnostic Systems, Sparks, Maryland). Isolated colonies were transferred to individual CII+H+1 agar plates and grown for 72-96 hours at 37°C until lawns were formed. Once the lawns were inspected for homogeneity, they were scraped into tubes with Mueller-Hinton broth (BD Diagnostic Systems) supplemented with 0.025% iron pyrophosphate, 0.1% glucose, and 2% IsoVitaleX™ (BD Diagnostic Systems), at pH 7.0 (Baker et al., 1985), with 10% sucrose for long-term storage at ≤70°C (Conlan & North, 1992). Bacterial viability and titer for this trial were verified once the stock was thawed using dilution plating on CII+H+1 agar. The stock was diluted in Dulbecco’s phosphate-buffered saline (Sigma, St. Louis, Missouri) prior to inoculation onto the material coupons.

**Test Materials**

Seven materials commonly found in laboratories and BSCs were used as test surfaces: acrylic, glass, polyamide, polyethylene, polypropylene, silicone rubber, and stainless steel. Samples of each test material were cut from a larger piece of the representative materials to form 2.0 cm x 5.0 cm coupons with the exception of glass (microscope slides; VWR International, Inc., West Chester, Pennsylvania) and stainless steel (1.9 cm x 7.5 cm coupons). A visual inspection of the physical integrity and appearance of the test material coupons was performed before and after decontamination to detect any damage to the test materials. All coupons were sterilized by autoclaving at 121°C for 20 minutes prior to testing.

**Decontamination Procedure**

All testing was performed under BSL-3 conditions. Each coupon was laid flat on a wire rack with support fencing (Rogers et al., 2005) in a BSC III, and contaminated with a suspension of approximately 7.0 x 10⁶ CFU of *F. tularensis* Schu S4. This inoculation consisted of placing two rows of five droplets (10 μL/droplet) using a multi-channel micropipette (Figure 1). The droplets were allowed to dry for one hour prior to decontamination. For each test material, three inoculated coupons and one blank (not inoculated) were used for decontamination. In parallel, two sets of inoculated coupons (N=3/material) and blanks (N=1/material) were used as controls. The first set was used to control for any potential decrease in microorganism viability during the one-hour drying time. The second set of control coupons were maintained in a separate, isolated BSC III to control for any potential decrease in microorganism viability during the entire three-hour decontamination run.

Biological indicators (BI) containing *Bacillus subtilis* (ATCC 19659) and *Geobacillus stearothermophilus* (ATCC 12980) spores on stainless steel disks sealed in Tyvek pouches (Apex Laboratories, Inc., Apex, North Carolina) and spore strips (SS) containing *B. atrophaeus* (ATCC 9372) spores on filter paper strips sealed in glassine envelopes (Raven Biological Laboratories, Omaha, NE) were also used to evaluate decontamination as previously described (Rogers et al., 2005; Rogers et al., 2007). These BI and SS contained approximately 1 x 10⁷ spores per indicator. Three of each BI and SS were subjected to hydrogen peroxide fumigation and three of each BI and SS not subjected to decontamination were used as positive controls.

The BSC III used for the decontamination run was approximately 128 ft³ (approximately 3,625 L). An Amisco VHP® Generator Series 1000 (STERIS Corporation, Mentor, Ohio) was connected to the BSC Class III cabinet and used for the hydrogen peroxide fumigation. The decontamination process consisted of dehumidification (four minutes), conditioning (20 minutes), sterilization (120 minutes), and aeration (30 minutes) phases, which were controlled by the hydrogen peroxide generator. The
injection rates during the conditioning and sterilization phases were set at 3.0 g/L and 3.1 g/L, respectively. Prior to initiating the decontamination run, the temperature and relative humidity inside the BSC III were measured using a NIST-traceable thermometer/hygrometer (VWR International, Inc.).

**Sample Processing**

Following the one-hour drying time, one set of control coupons (inoculated and blank coupons for each material type) were placed into 50 mL conical tubes containing 10 mL of Mueller-Hinton broth containing 0.025% iron pyrophosphate, 0.1% glucose, and 2% IsoVitaleX™. At the end of the decontamination run, the second set of control and decontaminated coupons inoculated with *F. tularensis* (and corresponding blanks) were placed into 50 mL conical tubes containing 10 mL of Mueller-Hinton broth supplemented with 0.025% iron pyrophosphate, 0.1% glucose, and 2% IsoVitaleX™. The BI and SS were placed into 50 mL conical tubes containing 10 mL of tryptic soy broth (Remel, Lexena, Kansas). All tubes containing coupons were gently agitated and incubated at 37°C for *F. tularensis*, *B. subtilis*, and *B. atrophaeus*, and at 55-60°C for *G. stearothermophilus*. The cultures were visually inspected for growth at one and four days of incubation. This one- to four-day time frame was chosen based on previous qualitative growth studies evaluating the efficacy of antimicrobial agents against *F. tularensis* (Baker et al., 1985; Brown et al., 2004).

**Results**

The decontamination run lasted approximately three hours from start to finish; no physical damage was observed for the hydrogen peroxide-exposed coupons upon visual inspection. The starting temperature and relative humidity were approximately 20°C and 40%, respectively. For all coupon materials, the one-hour drying controls and unexposed decontamination controls exhibited positive growth at one and four days (Table 1). VHP fumigation of inoculated coupons in the 3,625 L BSC Class III resulted in the complete inactivation of *F. tularensis* as demonstrated by the lack of viable growth in liquid cultures at one and four days (Table 1). All control and decontamination blanks were negative for growth (Table 1).

For all BI and SS exposed to VHP, no growth was observed as determined by the lack of visibly cloudy liquid cultures at one and four days post-exposure (Table 2). All of the unexposed BI and SS exhibited growth as determined by the presence of visibly cloudy liquid cultures at both one and four days (Table 2).

**Discussion**

As a fumigant, hydrogen peroxide exhibits decontamination efficacy against a wide range of microorganisms (Klapes & Vesley, 1990; Heckert et al., 1997; French et al., 2004; Johnston et al., 2005; Rogers et al., 2005). Hydrogen peroxide is considered less toxic than other...
fumigants such as chlorine dioxide, ethylene oxide, methyl bromide, and formaldehyde, and is neutralized by catalytic breakdown into water and oxygen. Moreover, hydrogen peroxide fumigation does not appear to damage materials or sensitive equipment (French et al., 2004; Rogers et al., 2005). Therefore, hydrogen peroxide fumigation has been used for decontaminating laboratories and laboratory equipment (Klapes & Vesley, 1990; Kahnert et al., 2005; Hall et al., 2007), medically-implanted wires (Fichet et al., 2004) and medical equipment (Andersen et al., 2006), hospital rooms (French et al., 2004), biological safety cabinets (Hillman, 2004), and animal holding rooms (Krause et al., 2001).

The present study demonstrates that \textit{F. tularensis} Schu S4 is inactivated on various material surfaces when exposed to VHP fumigation in a BSC III. This fumigation did not promote observable physical damage to the test material surfaces, which is similar to previous observations (Rogers et al., 2005). In parallel, BI containing \textit{B. subtilis} and \textit{G. stearothermophilus} spores and \textit{B. atrophaeus}

### Table 1

Decontamination efficacy of \textit{Francisella tularensis} Schu S4 following hydrogen peroxide fumigation on seven different test materials (N=3/material).

<table>
<thead>
<tr>
<th>Treatment/Test Material</th>
<th>No. Tested (No. Positive)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Peroxide Fumigation*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylic</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Glass</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Polyamide</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Silicone Rubber</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>No Fumigation (1 hr drying controls)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylic</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Glass</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Polyamide</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Silicone Rubber</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>No Fumigation (decontamination controls)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylic</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Glass</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Polyamide</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Polypropylene</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Silicone Rubber</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

*All blanks (not inoculated) for each material were negative for growth.

### Table 2

Decontamination efficacy of biological indicators and spore strips following hydrogen peroxide fumigation.

<table>
<thead>
<tr>
<th>Treatment/Test Material</th>
<th>No. Tested (No. Positive)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen Peroxide Fumigation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{B. subtilis} ATCC 19659 Biological Indicator</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>\textit{G. stearothermophilus} ATCC 12980 Biological Indicator</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>\textit{B. atrophaeus} ATCC 9372 Spore Strip</td>
<td>3 (0)</td>
<td>3 (0)</td>
</tr>
<tr>
<td>No Fumigation (decontamination controls)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{B. subtilis} ATCC 19659 Biological Indicator</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>\textit{G. stearothermophilus} ATCC 12980 Biological Indicator</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>\textit{B. atrophaeus} ATCC 9372 Spore Strip</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>
SS were also completely inactivated by VHP fumigation. The growth assessments of various BI and SS are often used to qualitatively evaluate decontamination performance (Heckert et al., 1997; Sigwarth & Stark, 2003; French et al., 2004; Hillman, 2004; Johnston et al., 2005; Rogers et al., 2005; Rogers et al., 2007). In the present study, this growth assessment demonstrated that both BI and SS exposed to VHP fumigation were completely inactivated as demonstrated by no observed growth in the liquid cultures at one and four days. These results suggest the VHP fumigation of the BSC III inactivated bacterial spores to the level of at least 6-logs, supporting the use of BI or SS during hydrogen peroxide fumigation of F. tularensis contaminated materials to qualify decontamination runs.

In this study, the approach used to evaluate the effectiveness of VHP fumigation against F. tularensis was a qualitative growth/no growth assessment. Such evaluations do not provide quantitative values, such as a log reduction that can be useful in assessing the degree of decontamination, or enabling statistical comparisons. However, this qualitative assessment was performed for two reasons. First, F. tularensis Schu S4 can persist in the environment for extended periods of time (Anda et al., 2001; Feldman et al., 2001; Abd et al., 2003; Feldman, 2003), and the median intracutaneous and respiratory infectious doses are less than 10 cells (Saslaw & Carlisle, 1969), making it important to completely inactivate F. tularensis by decontamination. The persistence on material surfaces was demonstrated previously where approximately 1 x 10^7 F. tularensis cells deposited on metal surfaces remained viable for days, depending on the relative humidity, with the fastest decline of 4-logs observed at one and a half days under 100% relative humidity (Wilkinson, 1966). Consistent with previous observations, our results showed that viable F. tularensis remained on the control coupons after the one-hour drying time as well as during the three-hour decontamination run. Related to persistence, the aging of aerosols at 24°C under 85% relative humidity affects the infectivity of F. tularensis Schu S4 in humans and non-human primates where low inhaled bacterial concentrations (150-750 viable cells) aged for 60 minutes exhibited 75% infectivity of exposed humans, while no humans were infected when the bacteria were aged 120 minutes or longer (Sawyer et al., 1966). However, F. tularensis at concentrations of 7,500-50,000 viable cells were infectious when aged for up to 180 minutes (Sawyer et al., 1966). Therefore, based on the worst case scenario of a 4-log decrease within one and a half days (Wilkinson et al., 1966), it is possible that with a starting inoculum of approximately 1 x 10^8 CFU F. tularensis in the present study, there may be at least 10,000 viable CFU remaining on the contaminated surfaces, which could potentially serve as a biological hazard requiring decontamination. However, this is only an assumption based on published inactivation rates, and further studies would be needed to quantitatively determine any loss in titer of F. tularensis Schu S4 on the test materials after drying for one hour.

Secondly, qualitative growth/no growth assessment can potentially provide a better estimate of complete inactivation compared to test material extraction and dilution plating. The extraction and plating procedures have been used previously to provide quantitative log-reduction values for decontaminating B. anthracis and surrogate spores using fumigants (Rogers et al., 2005; Rogers et al., 2007). This extraction and dilution plating approach typically yields less than 100% recovery of microorganisms from test material coupons, which is dependent on material porosity and complexity. Thus, the data derived from this type of analysis often provides a conservative estimate of microorganism decontamination at least to the level of recoverable, detectable microorganisms in the unexposed controls. However, this approach in log reduction determinations may be suitable for discerning effective decontamination of organisms such as B. anthracis where the estimated LD50 for pulmonary anthrax in humans is at least 8,000 spores (Inglesby et al., 2002). This is much higher than the reported median infectious and lethal dose concentrations of F. tularensis in humans (Carlisle, 1969). Complete inactivation (i.e., no growth) of F. tularensis may be necessary for effective decontamination remediation since such a requirement is the current criterion for remediation of a B. anthracis-contaminated site (Canter, 2005).

This study provides information for using hydrogen peroxide fumigation as an alternative approach to the current accepted method of 10% household bleach followed by 70% alcohol for decontamination of virulent F. tularensis. In this study, there were additional variables not evaluated that include other test material types such as porous surfaces, effects of relative humidity and temperature, and various methods of applying F. tularensis to test materials. Further studies are needed to address these parameters with respect to hydrogen peroxide decontamination of virulent F. tularensis.

Acknowledgments

We thank Robert Davenport for his technical assistance. This study was funded by the Battelle Biomedical Research Center.

References


Emerging Infectious Diseases, 7, 575-582.


Stability of Viral Pathogens in the Laboratory Environment

Hector N. Valtierra
San Diego Community College District, San Diego, California

Abstract

Knowledge of the stability of pathogens in the environment is part of a comprehensive biological risk assessment. The inherent nature of laboratory equipment and laboratory procedures to create aerosolized droplets of infectious agents, with subsequent deposition of these particles, provides opportunities to contaminate fomites such as laboratory equipment and personal items. Vivaria create an increased level of virus amplification and fomite contamination as the dried waste of virus-infected research animals can become aerosolized, or transmitted through direct contact. This paper provides a review of studies in which several species of virus have been allowed to dry on porous, or nonporous substrates, and analyzed at timed intervals to determine the ranges over which these viruses remain stable at room temperature as measured by their viability to infect cell cultures or research animals. The research shows that some viruses retain viability for up to a month or longer in the laboratory environment. This review will aid investigators and biosafety professionals in both risk assessment as well as decontamination efforts.

Keywords
Virology, viral stability, biosafety, environmental stability, biosecurity, risk assessment, biocontainment, disinfectant, decontamination

Introduction

Biological risk assessment, as defined in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) (5th Edition), is a process used to identify the following: the hazardous characteristics of a known infectious, or potentially infectious agent or material; the activities that can result in a person’s exposure to an agent; the likelihood that such exposure will cause a laboratory-associated infection; and the probable consequences of such an infection. Both the BMBL and the World Health Organization’s (WHO) Laboratory Biosafety Manual (3rd Edition) include pathogen stability in the environment as an essential component in conducting risk assessments. Although much is known about the ability of bacterial pathogens to survive for great lengths of time in various environments in dormant states, several studies on viral pathogens have shown a remarkable ability of these agents to remain viable in the laboratory and vivarium environment.

Previous reports have focused on the infectivity of aerosolized virus (Peters et al., 1996) and viral survival under general environmental conditions (Pirtle & Beran, 1991). In addition, reviews concerning a select few human pathogenic viruses under laboratory conditions have been considered (Mahl & Sadler, 1975; Sattar & Springthorpe, 1996; Kramer et al., 2006). The objective of this review is to examine the stability of viruses on surfaces with respect to conditions typically found within the context of the laboratory environment. Virus studies pertinent to the field of laboratory biosafety have been tabulated if they were conducted at 25°C (plus or minus 5°C) and a relative humidity of 50% (plus or minus 10%). Typically, these viruses were deposited in buffer onto porous (cloth or gauze) or non-porous (metal or glass) objects and allowed to dry at room temperature at 50% relative humidity. At measured time points from this initial deposition, samples were re-suspended in buffer, and either inoculated onto cells in Petri dishes, or in some cases, directly into animals to determine the viability of the virus to cause cytopathic effect, or infection. The reader is invited to review primary literature on a case-by-case basis to determine the specific conditions for each virus, suspension buffer, and cell type used when applying data toward the research group’s own risk assessment.

Viruses included in this review are bloodborne (such as HIV and Hepatitis B), possess the potential to be used as vectors (such as HIV, adenovirus, and vaccinia), viruses transmitted readily via the aerosol route (such as influenza virus and coronavirus), and/or possess special considerations with regard to vivaria.

Bloodborne Pathogens

In 1991, the U.S. Department of Labor Occupational Safety and Health Administration (OSHA) set forth Regulations (29 Code of Federal Regulations), Bloodborne Pathogens Standard 1910.1030 to protect workers at risk of coming into contact with potentially infectious pathogens found in blood and body fluids. These pathogens include, but are not limited to, hepatitis B virus, hepatitis C, and human immunodeficiency virus (HIV) (Table 1). Viruses present in blood and body fluids are surrounded by a high organic load carrying a high potential to remain viable for long durations.

Lentiviral vectors include: human vectors based upon HIV, and vectors from non-human sources based upon feline immunodeficiency virus, simian immunodeficiency virus, or equine infectious anemia virus, as well as hybrid HIV/SIV (SHIV) vectors. According to the Recombinant DNA Advisory Committee Guidance Document, either BL2 containment, or enhanced BL2 containment, is appropriate in the laboratory setting for research involving...
the use of advanced lentivirus vector systems possessing multiple safety features, and that segregate vector and packaging functions onto four or more plasmids (RAC, 2006). Researchers will continue to use HIV and retroviral vectors as these viruses allow for the incorporation of retroviral nucleic acid into the cellular genome.

**Adenovirus**

The family Adenoviridae includes double-stranded DNA viruses lacking a lipid envelope. Adenoviral vectors are widely used in *in vitro* and *in vivo* gene transfer studies in both animal models and clinical trials (Chuah et al., 2003). Adenoviruses should not be confused with aden-associated viruses, a virus in the family Paroviridae, since both of these viruses are used in gene transfer studies.

Adenoviruses and their cognate vectors differ in that the parental virus can cause a productive infection associated with viral replication, whereas adenoviral vectors are replication impaired (Chuah et al., 2003). Adenoviruses remain a cause of respiratory infections in children and is readily transmitted in daycare centers. Given the prolonged viability from one week to 12 weeks (Table 2), researchers using viruses in this family should consider employing disinfectants suitable for these membrane-lacking viruses.

**Influenza Virus**

Members of the family Orthomyxoviridae are enveloped viruses containing genomic RNA in eight segments. Although seasonal influenza causes significant morbidity and mortality, reconstructed 1918 influenza and highly pathogenic avian influenza pose special cases with regard to biocontainment procedures. Of special note is the viability for up to six days on steel and cotton fabric for low pathogenicity avian influenza (Tiwari et al., 2006), an important consideration for livestock, poultry, and human pathogen research, considering that different influenza strains have the ability to readily cross from species to species (Table 3).

The virus’ RNA genome is highly susceptible to mutation by virtue of being constructed by the viral RNA polymerase, in addition to the unique constellation of eight RNA strands that are inherited from different parental strains. The wide range of viability reported for environmental influenza strains may be attributed to the disparate biological properties expressed by the unique combination of genes found in each strain. A recent study of four different strains on bank notes showed a viability ranging from two hours to five days (Thomas et al., 2007). As such, each strain of influenza being studied must undergo a thorough risk assessment prior to research.

**Vaccinia Virus**

The family Poxviridae contains a variety of vector candidates, from a replicative virus exhibiting some virulence in animal models (such as wild-type vaccinia virus) to a non-replicative virus with no detectable virulence even when tested in severely immunocompromised animals (such as highly attenuated vaccinia strains or canarypox virus) (Vanderplasscen & Pastoret, 2003). As such,

### Table 1

Environmental stability studies on viral bloodborne pathogens. Hepatitis B virus is a lipid enveloped member of family Hepadnaviridae and has a partially double-stranded DNA genome. HIV is a lipid-enveloped member of the family Retroviridae and has two genomic RNA strands.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Survival Time</th>
<th>Substrate</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T-cell Lymphotropic virus (HTLV-I-III-M)</td>
<td>&gt;3 days</td>
<td>culture plates</td>
<td>Resnick et al., 1986</td>
</tr>
<tr>
<td>HIV-1 HTLV-IIB strain</td>
<td>&gt;34 days</td>
<td>glass slide</td>
<td>Tjotta et al., 1991</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>&gt;7 days</td>
<td>silynosed screwcap tubes</td>
<td>Bond et al., 1981</td>
</tr>
<tr>
<td>HIV-1 (RF strain)</td>
<td>4 - 8 wks</td>
<td>glass cover slide</td>
<td>van Bueren et al., 1994</td>
</tr>
</tbody>
</table>

### Table 2

Environmental stability studies on Adenovirus.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Survival Time</th>
<th>Substrate</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus 19</td>
<td>8 - 10 days</td>
<td>paper, cloth</td>
<td>Nauheim et al., 1990</td>
</tr>
<tr>
<td>Adenovirus 3</td>
<td>10 days</td>
<td>paper gum (dextrin)</td>
<td>Selwyn, 1965</td>
</tr>
<tr>
<td>Human Adenovirus type 3</td>
<td>&gt;9 days</td>
<td>polystyrene Petri dish</td>
<td>Rabenau et al., 2005</td>
</tr>
<tr>
<td>Adenovirus 19</td>
<td>35 days</td>
<td>plastic</td>
<td>Nauheim et al., 1990</td>
</tr>
<tr>
<td>Adenovirus-2</td>
<td>8 - 12 wks</td>
<td>glass slides</td>
<td>Mahl et al., 1975</td>
</tr>
</tbody>
</table>
there have been several incidents of Laboratory-Associated Infections (LAIs) reported within the last 10 years (Rupprecht et al., 2001; Mempel et al., 2003; Moussetché et al., 2003; Lewis et al., 2006). Due to the highly infectious nature of this virus, the Advisory Committee on Immunization Practices guidelines recommends the use of vaccinia vaccine to protect three classes of people susceptible to infection: 1) laboratory workers working with nonvariola Orthopoxviruses (e.g., vaccinia and monkeypox); 2) persons working in animal care areas where studies with Orthopoxviruses are being conducted; and 3) health-care workers involved in clinical trials using recombinant vaccinia virus vaccines (ACIP, 2001). The one study found in the literature search indicates the virus has a robust survival time despite being an enveloped virus (Table 4) (Mahl et al., 1975).

As in the case of HIV, a rich organic load sustains viability of the smallpox virus. Research has shown that the smallpox virus is stable in crusts of patients for two to four months depending on the humidity of the stored material (Essbauer et al., 2007).

Zoonotic Viruses

Viruses used in animal research studies have the ability to be amplified within the laboratory animal to high concentrations. Viruses may be introduced into the animal through experimental protocol or through unintentional infection from feral animals in the animal facility due to faulty pest management systems. In the first case, viruses may be inherently infectious to the laboratory animal, attenuated, or have virulence enhanced by experimental methods. An important consideration is that “attenuated” does not necessarily mean “not infectious.” For example, CDC describes the Ankara strain of MVA as highly attenuated—but recommends Biosafety Level 2 containment (ACIP, 2001). In the latter case, unintended virus infections may disrupt experiments by causing morbidity or mortality of laboratory animals. For these reasons, several different viruses seen in animal populations are provided (Table 5).

The potential for viruses infecting rodents (e.g., rat virus) to remain viable for up to seven weeks on bedding, and up to five weeks on plastic surfaces, indicates that fomites and equipment must be decontaminated, or disposed of properly in order to halt viral transmission in vivaria. The rat coronavirus was viable up to three days on plastic plates. Avian metapneumonovirus and low pathogenicity avian influenza display survival up to six days. Heightened awareness of biosecurity and biosafety measures as they pertain to the use of small mammals in vaccine trials, or biocontainment practices in the laboratory are therefore indicated.

SARS Virus

The severe acute respiratory syndrome (SARS) coronavirus (CoV) appeared in China in 2002 and became a pandemic within months. SARS-CoV ultimately infected more than 8,000 people in late 2002 and 2003. Researchers scrambled to investigate this unknown virus and the genome was sequenced within one year of emergence.

Coronaviruses exhibit a moderate ability to retain viability on different substrates (Table 6). This family of viruses possesses a lipid envelope and a plus-sense single-strand RNA genome. The highly infectious nature of the disease, coupled with inappropriate laboratory standards and practices led to at least three instances of LAIs in Singapore, Taiwan and mainland China in September 2003, December 2003, and April 2004 (Lim et al., 2006).

### Table 3

Environmental stability studies on Influenza virus.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Survival Time</th>
<th>Substrate</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A (FR8 strain)</td>
<td>&gt;3 hours</td>
<td>stainless steel</td>
<td>Sattar et al., 2006</td>
</tr>
<tr>
<td>Influenza B</td>
<td>48 hours</td>
<td>stainless steel</td>
<td>Bean et al., 1982</td>
</tr>
<tr>
<td>Influenza A (H1N1)</td>
<td>72 hours</td>
<td>stainless steel</td>
<td>Bean et al., 1982</td>
</tr>
<tr>
<td>Low Pathogenicity Avian Influenza</td>
<td>~6 days</td>
<td>steel</td>
<td>Tiwari et al., 2006</td>
</tr>
<tr>
<td>Influenza A (FR8 strain)</td>
<td>&gt; 3 days</td>
<td>cloth sheet</td>
<td>Edward et al., 1941</td>
</tr>
<tr>
<td>Low Pathogenicity Avian Influenza</td>
<td>~6 days</td>
<td>cotton fabric</td>
<td>Tiwari et al., 2006</td>
</tr>
<tr>
<td>Low Pathogenicity Avian Influenza</td>
<td>~6 days</td>
<td>plastic</td>
<td>Tiwari et al., 2006</td>
</tr>
<tr>
<td>Influenza A (FR8 strain)</td>
<td>4 wks</td>
<td>glass sides</td>
<td>Edward et al., 1941</td>
</tr>
</tbody>
</table>

### Table 4

Environmental stability study on Vaccinia virus, a lipid-enveloped virus with a DNA genome.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Survival Time</th>
<th>Substrate</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinia virus (Lederle strain)</td>
<td>4 - 5 wks</td>
<td>glass sides</td>
<td>Mahl et al., 1975</td>
</tr>
</tbody>
</table>
Herpes Virus and B Virus

A special case of zoonotic virus warrants its own category because of the various uses of many species of primates in laboratory research. In 1932, Dr. W. B., a 29-year old researcher was engaged in experimental work on poliomyelitis when he was bitten on the dorsum of the left ring and little fingers by an apparently normal Macaca rhesus monkey. Sixteen days later he died (Sabin & Wright, 1934). Since this event, there have been several other LAI reports of B virus infection with some of these cases leading to death (Holmes et al., 1990; Ostrowski et al., 1998). B virus, or Cercopithecine herpesvirus [CHV-1], is a member of the family Herpesviridae and, as such, is enveloped with a linear double-strand DNA that becomes circular in the host cell.

Members of the B Virus Working Group met at the Centers for Disease Control and Prevention (CDC) in January 1999 and formulated two recommendations. First, animal workers who care for macaques should be informed of the biohazards associated with these monkeys and the importance of notifying their supervisors and occupational health care personnel of all bites, scratches, and mucocutaneous exposures. Secondly, macaques should be treated as if they are seropositive for B virus regardless of their origin (Cohen et al., 2002). The majority of studies on Herpesviridae species indicate a decreased viability on porous and non-porous substrates despite its DNA genome (Table 7).

Discussion

Of the 177 pathogenic species regarded as emerging or re-emerging pathogens, the majority are viruses (77 species), followed by bacteria (54 species), fungi (22 species), protozoa (14 species), and helminths (10 species) (Zessin, 2006). Viruses will continue to be studied in the laboratory as researchers study new ways to ameliorate viral disease in humans, livestock, and plants. In addition to basic biological research, viruses are studied for vaccine and gene therapy development. Viruses can also be found in chronic disease research that address cancers caused by papillomavirus and herpesvirus.

The studies in this review indicate varying viral stability on porous (cloth) and non-porous surfaces.
(metal, glass, plastic) at typical laboratory relative humidity and temperature values. Within virus species, the retention of viability on metal surfaces is less than on glass or cloth substrates. This may indicate a possible virucidal mechanism of metals, as suggested by Bardell (1994), and supported by different survival times seen for the influenza virus on copper and steel (Noyce et al., 2007). However, viability on surfaces is only part of the viral environmental stability equation. One must also look at viral stability while in aerosolized droplets. At least two viruses in this review, SARS and influenza, are readily transmitted by the respiratory route. Taken together, aerosolized and surface-laden viruses represent significant potential sources of infection in the laboratory environment.

As with all components of an institute’s successful biological safety plan, training remains at the forefront of educating laboratorians how to protect themselves, as well as taking precautions to avoid infecting coworkers, including custodial staff. Presenting viral environmental stability studies to laboratorians raises their awareness that some viruses have the ability to survive for days or weeks in the laboratory environment.

Further virus stability studies will provide a more complete picture of other factors that influence environmental stability. In particular, little, or no research has been conducted on the stability of viruses affecting plants, an important consideration in agricultural bioccontainment. Potential uses for these experiments, as suggested by Stuart and Wilkening (2005) include understanding degradation times of potential biological weapons with implications for time management during a terrorism response. Similarly, understanding the parameters required for virus breakdown in a contaminated lab, clinic, or vivarium will enable the development and implementation of effective strategies to alter environmental conditions such as relative humidity and temperature, potentially reducing costly decontamination procedures.

### References


---

**Table 7**

Environmental stability studies on Herpesvirus.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Survival Time</th>
<th>Substrate</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes Simplex Virus-1, strain F</td>
<td>&lt;2 hours</td>
<td>coin penny</td>
<td>Bardell, 1994</td>
</tr>
<tr>
<td>Herpes Simplex Virus-1, strain F</td>
<td>&gt;2 hours</td>
<td>chrome-plated tap handle</td>
<td>Bardell, 1990</td>
</tr>
<tr>
<td>Herpes Simplex Virus-1 and -2</td>
<td>18 - 24 hours</td>
<td>speculum</td>
<td>Larson et al., 1985</td>
</tr>
<tr>
<td>Herpes Simplex Virus-1, strain F</td>
<td>&gt;2 hours</td>
<td>glass</td>
<td>Bardell, 1994</td>
</tr>
<tr>
<td>Herpes Simplex Virus-1, strain F</td>
<td>&gt;2 hours</td>
<td>plastic doorknob</td>
<td>Bardell, 1990</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>8 hours</td>
<td>plexiglass</td>
<td>Feix, 1985</td>
</tr>
<tr>
<td>Herpes Simplex Virus-1</td>
<td>1 d - 1 wk</td>
<td>glass slides</td>
<td>Mahl et al., 1975</td>
</tr>
<tr>
<td>Herpes Simplex Virus-1 (strain McIntyre)</td>
<td>3 days</td>
<td>polystyrene Petri dish</td>
<td>Rabenau et al., 2005</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>2 hours</td>
<td>blanket</td>
<td>Feix, 1985</td>
</tr>
<tr>
<td>Herpes Simplex Virus</td>
<td>&gt;3 days</td>
<td>gauze</td>
<td>Larson et al., 1985</td>
</tr>
</tbody>
</table>
Articles


Rapid and Biologically Safe Procedures for the Evaluation of Antigen-Specific T Cell Response to Microbial Pathogens That May Be Used in the BSL-3 and BSL-4 Environment

Chiara Agrati, Ilaria Volpi, Federico Martini, Cristina Gioia, Concetta Castilletti, Giuseppe Ippolito, Maria Rosaria Capobianchi, and Fabrizio Poccia

National Institute for Infectious Diseases “Lazzaro Spallanzani”-IRCCS, Via Portuense, 292, Rome, Italy

Abstract

T cell response precedes serological response, and is more feasible than pathogen detection by molecular tools. Thus, monitoring T cell response to class-A pathogens may allow for a timely treatment and a correct handling of patients containment issues, after exposure to potentially lethal agents, such as biological threat, or emerging pathogens. In this paper, we describe a procedure for the evaluation of T cell response to microbial pathogens, including class-A pathogens in BSL-3 and BSL-4 equipped laboratories.

Introduction

The class A pathogen list includes the most dangerous agents potentially used as bioweapons (Centers for Disease Control and Prevention [CDC], 2006b). The timely handling of possibly contaminated samples without previous knowledge about the infecting agent should be performed in BSL-3 and BSL-4 laboratories equipped with adequate facilities (CDC, 2006d). Current laboratory tests for class A pathogens include: serologic, molecular, and culture-based methods (CDC, 2006c; CDC, 2006a; Fasanella et al., 2003; Mahanty et al., 1999). PCR-based detection of nucleic acids is a good method to detect the presence of pathogens in samples, but the results are not always reliable, and the procedure requires specially equipped laboratories and specific technical expertise. Both laboratory organization and technical knowledge are necessary to ensure reliable results and false-positive contamination problems (Kwok & Higuchi, 1989; Lo & Chan, 2006). Specific antibody detection by an Enzyme-Linked ImmunoSorbent Assay (ELISA), or immunofluorescence assay is more easily applied, and allows for the identification of both infected and exposed persons; however, a detectable antibody response may take weeks to develop. A T cell response to an infectious agent, in contrast, occurs very quickly, thus allowing for earlier detection of a pathogen-specific immune response based on the presence of circulating effector T cells (Gioia et al., 2005). With this method, it could be possible to identify exposed and infected persons in a more timely manner, allowing for early treatment and correct handling of patients containment issues following exposure to potentially lethal agents, such as biological agents or emerging pathogens. Moreover, a quantitative and qualitative analysis of CD4 and CD8 T cell-mediated responses may help to understand the role of cell-mediated immunity against class A pathogens. A T cell array flow cytometry technique, based on exposure of peripheral blood mononuclear cells (PBMC) to antigens, was recently evaluated by Gioia that allowed for the characterization of an antigen-specific T cell profile, based on the detection of intracellular interferon (IFN)-γ production (Gioia et al., 2005; Poccia et al., 2003). Unfortunately, this technique is not easily applied within the BSL-3 and BSL-4 environment. To overcome the issue of working with high-risk pathogens, an effective inactivation measure was developed to allow samples to be handled in a BLS-2 laboratory. Paraformaldehyde (PFA) has been widely used for the chemical inactivation of high threat pathogens as it is known to not alter the antigenic, scatter and fluorescence properties of the cells (Lal et al., 1988). The recommended procedures for the PFA inactivation of class A pathogens are shown in Table 1 (CDC 2006d; CDC 2006a; Flick et al., 2003; Mahanty et al., 1999; Stroher et al., 2001). These procedures can be subdivided into two main general protocols: a protocol using a < 30 minutes of PFA inactivation (short exposure), and a procedure using overnight PFA inactivation (long exposure). More specifically, an easy and safe whole blood (WhBl) flow cytometry protocol designed according to BSL-3 and BSL-4 recommendations (WhBl-BSL-3/4 assay) has been designed. This protocol can be applied to class A pathogen-containing samples to allow for the detection of T cell response by phenotypic and functional characterization of pathogen-specific T cells. This WhBl-BSL-3/4 assay was validated in comparison with standard flow cytometry assay on whole blood (WhBl assay) and on purified peripheral blood mononuclear cells (PBMC).

To provide proof of concept for the suitability of the proposed protocol, it was necessary to observe cellular responses. At present, it is impossible to find any individual whose T cells are able to respond to Class-A antigens, while normal samples could make it very easy to set up a flow cytometry protocol including the requested inactivation step. We decided to use CMV antigens to set up the protocol by using normal donors tested for CMV whose T cells were known to have the ability to respond to
CMV antigens. In this way it was possible to validate a general inactivation procedure and maintain T cell activity readout, fulfilling the requested protocols for safe Class-A handling that can be applied in any situation.

Peripheral venous blood was collected in heparinized Vacutainer tubes (BD Biosciences) from normal controls known for CMV status by venipuncture. Blood samples were processed within our BSL-3 facility by diluting 1:2 in culture medium (RPMI-1640 medium containing 10% v/v heat-inactivated fetal calf serum [FCS]), and incubated overnight in 5% CO₂ atmosphere in the presence of the stimulating antigen(s), i.e., CMV lysate (2 μg/ml, Biowhittaker, East Rutherford, New Jersey); CMV p65 protein (2 μg/ml, Biodesign International, Saco, Maine); control peptide pool (enclosing CMV, Epstein Barr Virus, Influenza Virus peptides, 1 μg/ml, kindly provided by NIH) in the presence of the co-stimulatory antibodies anti-CD28 (1 μg/ml) and anti-CD49d (1 μg/ml, BD Biosciences). As additional controls, anti-CD28 (1 μg/ml) and anti-CD49d (1 μg/ml) cultures and anti-CD3 antibody (2 ng/ml, Biosource International, Camarillo, California) cultures were used. One hour after the start of the cultures, Brefeldin A (BFA, 10 μg/ml final, Serva, Heidelberg, Germany) was added to block intracellular transport and allow cytokine accumulation in the Golgi compartment. After overnight incubation, stimulated whole blood cultures were stained for surface markers (such as CD8, BD Biosciences), treated by FACS Lysing Solution, washed, inactivated by PFA 4% (according to short or long treatment protocols), and brought out of the BSL-3 facility. Fixed samples were incubated with IFN-γ-specific monoclonal antibody (IFN-γ-APC: IgG2b, clone 2573.11, BD Biosciences) according to the manufacturer’s instructions in labeling buffer (PBS containing 1% BSA, 0.1% sodium azide and 0.5% saponin) for 15 minutes at room temperature, and acquired by FACS-Calibur cytometer accordingly to standard procedures. In particular, lymphocytes were identified by morphologic parameters, and responder T cells were identified by CD3, CD8, and IFN-γ expression.

Results

Figure 1 shows the CD8 T cell response to different Ags preparations to include CMV lysate (panels D-F), CMV p65 protein (panels G-I) and control peptides (panels J-L) in a healthy subject previously characterized to be a CMV responder. The panels show the IFN-γ production (vertical axis) by CD8 T cells (horizontal axis) obtained by culturing responder’s PBMC with stimuli as described on the left column. The assay was performed by the WhBl-BSL-3/4 procedure (long inactivation protocol, right column) and compared to the standard WhBl (center column) and PBMC (left column) assays. Anti-CD3 and anti-CD28 plus anti-CD49d antibodies were used as controls (data not shown). The numbers in the upper-right quadrant represent the frequency of IFN-γ-producing CD8 T cells in the various experimental conditions. The evaluation of CMV-specific IFN-γ-producing CD8 T cells by the WhBl-BSL-3/4 procedure (Figure 1, panels F, I, L) was at least equivalent to those obtained by the standard procedures (PBMC: panels D, G, J; WhBl: panels E, H, K) for both whole CMV lysate, p65 and control peptides. Similar results were obtained in multiple experiments and are representative of at least three tests. The short-term (one hour) PFA treatment also gave similar results (data not shown). Interestingly, in both the standard WhBl and WhBl-BSL-3/4 procedures, slightly higher frequencies of antigen-specific CD8-T cells were detected, as compared to the PBMC assay, possibly reflecting a more efficient stimulation due to more physiological conditions.

The CD8 T cell response detection allows for identification of infected/exposed/immunized persons weeks before the occurrence of detectable antibody response (Gioia et al., 2005; Poccia et al., 2003), but it is not appli-
Antigen-specific T cell response. After in vitro stimulation with cytomegalovirus (CMV) antigen preparations (CMV lysate and p65) or with a positive control peptide pool (enclosing CMV, Epstein Barr Virus, Influenza Virus peptides), the frequency of IFN-\(\gamma\)-producing CD8 T cells was analyzed by flow cytometry. These experiments were performed on peripheral blood mononuclear cells (PBMC) (Panels A, D, G, J) and on whole blood (WhBl) both using standard (WhBl: Panels B, E, H, K) and BSL-3/4 (WhBl-BSL-3/4, Panels C, F, I, L) protocols.

Figure 1

The figure shows the results of the antigen-specific T cell response assay. The experiments were performed on PBMC or whole blood samples, with and without inactivation protocols. The panels illustrate the frequency of IFN-\(\gamma\)-producing CD8 T cells after stimulation with CMV lysate and p65, as well as with a control peptide pool. The panels demonstrate the variability in response across different protocols and sample types.

Cable for samples suspected to be infected with class A pathogens that must be handled in BSL-3 or BSL-4 facilities. We therefore standardized a flow cytometry procedure that by an inactivation step, performed according to recognized biosafety procedures, may be applied to CD8 T cell response to pathogen-related antigen preparation (such as viral lysates, proteins and peptides and, possibly, live viruses) without affecting its sensitivity. As T cell response to infectious agents occurs very early (days or weeks before the development of a serological response) this procedure may be used for the early detection of signs of exposure to a class A pathogen. Therefore, the availability of an easy-to-perform and safe assay that is able to reveal the presence of a T cell based pathogen-specific immune response may be a very helpful tool that can be used, in parallel with the detection of nucleic acids of the etiological agent, to reliably identify in the early stages class A pathogen-exposed and/or infected persons. Moreover, this procedure allows one to monitor the cell-mediated immunity in patients infected by class A pathogens.
Acknowledgements

The following reagent was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEF Control Peptide Pool (DAIDS) Catalogue number 9808.

This work was supported by grants from Italian Ministry of Health (Ricerca Corrente and PISEB), as well as by the European Commission funded project EuroNetP4.

References


Centers for Disease Control and Prevention (CDC). (2006c). Available at: www.bt.cdc.gov/labissues/#testing


Development and Validation of a Pilot Scale Enhanced Biosafety Level Two Containment for Performance Evaluation of Produce Disinfection Technologies

Joseph E. Sites¹, Paul N. Walker², Angela Burke¹, and Bassam A. Annous¹

¹U.S. Department of Agriculture, Agricultural Research Service, Wyndmoor, Pennsylvania, and
²Pennsylvania State University, University Park, Pennsylvania

Abstract

The development and validation of the Biosafety Level 2 (BSL-2) enhanced containment system located at the produce pilot plant facility of the U.S. Department of Agriculture—Eastern Regional Research Center is presented here. This multi-purpose containment is used to enclose pilot and pilot scale washing and sanitizing equipment for fruits and vegetables, or other decontamination equipment where aerosol generation is likely, and complete protection is required for researchers. This containment is operated under a negative pressure with all exhausted air (approximately eight containment air exchanges per hour) being passed in parallel through two hydrophobic HEPA filters. During operation, personnel are excluded from the containment, materials are introduced into and removed from the containment via pass-throughs, and equipment is operated via computer control and glove ports.

At the completion of any series of processing trials, vegetative bacterial cells remaining within the containment, and in the processing water, are inactivated by raising the internal temperature of the containment, and all contents, including equipment, processing water and waste water to sufficiently inactivate all bacterial cells, except spore formers, using industrial steam at atmospheric pressure. We have demonstrated the feasibility and safety of conducting multiple trials using pathogenic bacteria with pilot scale processing equipment within the containment, and then inactivating surviving vegetative bacterial cells with the steam-in-place proc-
ess. Furthermore, we validated pilot-scale surface pasteurization processing equipment capable of significantly reducing cell densities (in excess of 5 logs) of Salmonella on inoculated cantaloupes, using this BSL-2 enhanced containment system.

**Introduction**

The demand by consumers for fresh and fresh-cut fruits and vegetables has steadily increased due to the nutritious qualities associated with fresh produce, and the convenience of ready-to-eat fresh foods. This increased demand has resulted in increased per capita consumption of fresh fruits and vegetables in the U.S. (ERS, 2004). The market for packaged salads and fresh-cut fruits and vegetables is estimated at $10 to $12 billion annually (IFPA, 2004). Unfortunately, as produce consumption has increased in the U.S., so has the number of produce-related outbreaks of foodborne illnesses. Produce-related outbreaks accounted for 12% of all reported foodborne outbreaks in the 1990s compared to only 0.7% in the 1970s (USFDA, 2004; Sivapalasingam et al., 2004). The Centers for Disease Control and Prevention (CDC) reported that foodborne outbreaks associated with fresh produce doubled between the periods 1973 to 1987 and 1988 to 1992 (Buck et al., 2003). The continuing occurrence of such produce-related outbreaks may jeopardize further increases in per capita consumption due to lowered confidence in the microbial safety of the product by the consuming public. Such outbreaks can also have a significant economic impact on growers, processors, shippers, and food service companies. The current cost in medical care and decreased productivity due to foodborne illnesses has been estimated to be between $10 to $83 billion each year (USFDA, 2004).

Federal initiatives to improve the microbiological safety of fresh produce generally call for improvements in post-harvest washing and sanitizing procedures. Current technologies available for use by the produce industry result in no more than 2 to 3 log (99.99,9%) reductions in pathogen levels, and fall short of meeting the U.S. Food and Drug Administration’s (USFDA) target of 5 log (99,999%) reductions. Thus, more effective decontamination technologies and decontamination equipment are needed. Those interventions found to be effective in the laboratory should be scaled up in a pilot plant facility to facilitate the commercialization of the technology. However, human pathogens could not be introduced into a pilot plant, in its original configuration, for testing of pilot and commercial scale equipment, because of concerns for the safety of equipment operators, or other personnel, in the pilot plant area, and researchers found it necessary to use non-pathogenic surrogate organisms in place of the target pathogenic organism (Eblen et al., 2005). Such studies were inherently limited because surrogate organisms do not necessarily behave in a manner identical to that of their pathogenic counterparts. A pilot plant, designed to accommodate pilot scale experiments using human pathogens, was required to facilitate validation and commercialization of new technologies for decontaminating produce containing human pathogens.

To create such a facility, the Eastern Regional Research Center (ERRC), Food Safety Intervention Technologies Research Unit (FSIT) and the Pennsylvania State University (PSU) entered into a Cooperative Agreement to design and fabricate a novel Biosafety Level 2 (BSL-2) enhanced containment. This primary barrier would enclose pilot-scale, or commercial produce processing equipment, and could be operated and decontaminated without the need for special personnel protection clothing, or equipment. The selection of decontamination process was based on the following criteria: the process should be environmentally friendly; should penetrate into the core of motors and other inaccessible locations; should produce no antimicrobial chemical residues that might confound subsequent processing trials.

Pressurized steam was selected for decontaminating the BSL-2 enhanced chamber. The BSL-2 enhanced chamber was required to be capable of confining human pathogens on inoculated produce undergoing different decontamination wash treatments utilizing various pilot and commercial scale processing equipment, to facilitate research on new washing and sanitizing treatments that can benefit the fresh produce industry, while providing protection to the researchers conducting such studies. This manuscript describes the design, fabrication, and validation of a unique BSL-2 enhanced containment, pilot-scale produce washing equipment, and associated remote controls and decontamination systems.

**Materials and Methods**

**Containment Design**

The containment structure is a modular design to facilitate construction, transportation, and expansion by adding additional modules end-to-end. Currently, the system consists of two modules (Figures 1 and 2). Each module (approximately cubical) was constructed with a welded frame of square 304 stainless steel tubing. The tubes within each module, and each door were joined by welding so that the inside of all the tubes in each door, or module forms a single continuous enclosed cavity. These four frame cavities (two modules and two doors) are filled with pressurized steam (207 kPa) during the decontamination process, with the steam entering through a pipe at the top of the containment and the condensate leaving through a steam trap diagonally across the containment. This pressurized steam-filled frame is considered the key feature of the design, and it ensures that the corners, which would otherwise be difficult to thermally decontaminate, are fully heated. In fact, because pressurized steam is used, the temperatures of the corners become hotter than the inside of the containment where only
atmospheric pressure steam is used. The bottom, top, and doors of the containment are all enclosed with panels welded to the frame. The panels are folded at approximately 70° to minimize the stress between the frame and the panel due to differential expansion when the frame is filled with steam. The panels in the bottom are sloped so that the water drains to an edge point fitted with a drain pipe, which leads to the drain decontamination system. On the top of each module, the center area was fitted with a removable stainless steel panel clamped to the frame. The containment frame and metal panels were covered with sheets of aluminum-clad insulation on most outside surfaces to protect personnel from hot surfaces capable of causing burns, and to conserve energy.

The containment sides are constructed of clear Lexan polycarbonate sheets (Part #43029, U.S. Plastics, Lima, Ohio, USA), and are held in place with linear clamping mechanisms that permit thermal growth without inducing stress on the panels. These polycarbonate panels provide high visibility of the inside of the containment chamber during operation. Hollow silicone bulb seals, (Part #1129A3, McMaster-Carr, Dayton, New Jersey, USA) were attached to the ends of the modules to provide a seal between modules, and between the module and each door (Figure 2). Like the frame, the gaskets are also filled with pressurized steam (35 kPa) while the containment is in operation. This serves to prevent the possibility of leaking contaminated water that might accumulate on the gasket and would decontaminate the accumulated water due to the high temperature of the gasket.

Large produce processing equipment to be placed in the containment is normally outfitted with lockable casters. The equipment is rolled in through either of the large doors on the ends of the containment (Figure 1). Inside, the casters are supported and guided by two parallel channels (Figure 2). A movable perforated-metal grid walking platform is laid parallel to, and on either side of the equipment, to enhance personnel access to the equipment prior to closing the containment. Electric power and control circuits to the equipment are provided by weather-tight electrical cords and fittings, which enter the containment through weather-tight cord grips located in the removable ceiling panels of the containment.

The containment was designed to accommodate processing equipment that generates considerable heat and humidity. Therefore, a fan coil unit heat exchanger (Model #CB-12-CDW. Concepts and Designs Inc., Owatonna, Minnesota, USA) was installed to condense the water vapor and remove the heat (Figure 2). The all-stainless steel unit is suspended from the ceiling of one of the modules and is supplied with circulating chilled methanol solution available in the building. The conden-

---

**Figure 1**
BSL-2 Containment: A - Liquid Pass-Through (LPT); B - Aluminum Clad Insulation (typical); C - Computer Control Cabinet; D - Dry Pass-Through (DPT); E - HEPA Filters; F - Equipment Loading Doors

**Figure 2**
Axial View of BSL-2 Containment: A - Dry Pass-Through (DPT); B - Liquid Pass-Through (LPT); C - Fan Cooling Unit; D - Process Equipment; E - Silicone Gasket; F - Perforated Metal Grid Walking Platform; G - Equipment Support Channels; H - Drain Pan
sate drains from the unit discharge onto the floor of the containment. The reduction of humidity is particularly useful in minimizing obstruction of vision due to condensation on the windows.

During produce decontamination studies using the BSL-2 enhanced containment, produce samples can be moved in and out of the containment using dry and liquid pass-throughs, respectively, which are mounted in the side window panels (Figures 1 and 2). The dry pass-through (DPT) (Figure 3) is an air plenum passing through one of the window panels. The plenum is outfitted with a door on the inside, and a door on the outside, with a mechanical interlock system to ensure that both doors cannot be unintentionally opened at the same time, which might allow microbial contaminants to escape. The DPT contains a steam port and a drain for decontamination of the pass-through after each use, and condensate water is routed to the inside of the containment. The decontamination process of the DPT is independent of the decontamination process of the entire containment. This DPT was installed at an angle to facilitate the movement of materials into the containment. Also, a special stainless steel transfer container (0.6 m x 0.3 m x 0.3 m high) was designed (not shown) with one end door for loading inoculated produce through the DPT. The transfer container is filled with produce under a biosafety cabinet and then loaded into the DPT. The end door of the box is then unlatched, but is held closed by the latched inside door of the DPT. Next, the outside door of the pass-through is latched, allowing the inside door to be slowly opened, and allowing the produce to roll gently into the process equipment inside the containment.

The liquid pass-through (LPT) (Figure 4) is a tank divided by a wall, and is filled with approximately 90 liters of 500 ppm chlorine solution to prevent the escape of aerosols from the inside of the containment. It should be noted that the liquid level in the tank is higher on the inside of the containment than on the outside, owing to the vacuum inside. From the inside of the containment, samples are sealed in two Ziploc storage bags, and are submerged and held for 30 seconds in the chlorine solution, passed under the wall, and lifted out of the liquid on the outside of the containment. An integral collar made of square tubing surrounds the stainless steel tank and forms the surface for mating to the window. The structural angles on the inside surface of the windows help to complete the seal and carry the weight of the tank to the frame of the containment. The collar has a steam inlet at the top, and a condensate trap at the bottom, to help ensure that the interface is thoroughly heated during system decontamination. The tank’s manual drain valve is inside the containment so that the liquid may be drained to the containment pans.

The pan shaped containment floor collects liquid

**Figure 3**

Dry pass-through: Top lever operates inside door and bottom lever operates interlock (details not shown) so that only one door can be open at a time. Some components exaggerated to show detail.
and routes it to a drain (Figure 2). The liquid is a combination of condensate and water solutions from the process equipment that was splashed, spilled, or intentionally dumped. The drain liquids, and the drain itself, are decontaminated after each use of the containment by raising the liquid temperature through the use of a tube and shell heat exchanger (not shown) that is integral to the drain line.

During operation, the BSL-2 enhanced containment is kept under negative pressure using a regenerative blower (Figure 5). The path of air flow begins at the outside of the containment. Air enters the containment through any crevices in the structure, most notably where the windows are clamped to the frame. The filtered air then leaves the containment through a pipe attached to a top panel. The exhaust pipe leads to two sets of hydrophobic HEPA filters arranged in parallel, then to the blower. The discharged filtered air is ducted to the outside of the building. Each set of filters contains a coalescing 50.8 cm long Rigimesh Filter Element (Model #AB2RM7H4, Pall Corporation, East Hills, New York, USA) to reduce the amount of steam fog, followed by a 50.8 cm long hydrophobic PTFE (0.2 μm) HEPA filter (Model #AB2PFA7PVH4, Pall Corporation) to remove contaminants. The parallel nature of the filter sets allows for the use of multiple sets of filters to increase filtering capacity. When needed, each set can be isolated from the other set during operation by closing valves, decontaminated with steam, and then the filter housings opened to safely change filter elements.

Busch regenerative blower (Model #SB 0310 D2 HO EW XX, Dr. Ing. K. Busch Gmb H, Maulburg, Germany) is intentionally installed downstream of the filters, because the high pressure side of the blower is above atmospheric pressure, which means the air must be filtered before reaching this point to avoid contamination leaks through any of the crevices. In other words, the entire system, from the containment through the filters, is under negative pressure to avoid such leaks of contamination. The regenerative blower is powered by an electric motor controlled by a GE-Fuji frequency modulation controller (Model #6KP112307X4B1, GE Industrial Systems, Salem, Virginia, USA) with feedback from a Magnehelic Differential Pressure Indicating Transmitter Dwyer (Model #605-3, Dwyer Instruments, Inc., Michigan, Indiana, USA) in order to regulate the pressure inside the containment. As an added safeguard, this pressure indicating transmitter controls the steam valve; if the vacuum inside the containment falls to below 0.2 mm of Hg, the valve is closed.

The BSL-2 enhanced containment system and the processing equipment are simultaneously controlled by a personal desktop computer (Figure 2) with Lab View 7.1 software (National Instruments, Houston, Texas, USA). The computer system, which can be controlled remotely through a local area network, is located next to the containment unit.
Temperature Monitoring

Air and water temperatures at various locations within the containment and DPT were monitored and recorded using a data acquisition system consisting of Resistance Temperature Detectors (RTDs), smart transmitters (Model #AI-100 R-2, Accuteck, Hudson, Massachusetts, USA) with a 4-20 mA output, one data acquisition card (Model #NI-PCI-6024E, National Instruments). This system was calibrated with a high accuracy digital thermometer (Model #DP97, Omega Engineering, Stamford, Connecticut, USA).

Surface temperatures were measured with non-reversible temperature indicator strips Thermax 6ML-3 (Thermographic Measurement Ltd., Glenview, Illinois, USA), having a range of 65-93°C, with approximately 6°C increments, and an accuracy of ± 1°C, or with 30 AWG surface mounted fiberglass insulated thermocouples (Model E SA1XL-T-72, Omega Engineering).

Containment and Decontamination Process

Produce processing equipment, such as the dip tank, which is an experimental pilot scale produce cleaning device designed by the ERRC-PSU team that has a capacity of 235 liters, and is capable of operating at temperatures between 4-95°C, is placed inside the containment; the doors are closed with the gasket steam pressurized. The regenerative blower is then started. From this point, the profile of a typical cycle consisting of five main phases is shown in Figure 6.

Containment Phase—Section “A”: The regenerative blower creates a negative pressure (typically 0.25 cm water pressure) inside the containment that induces an inward airflow to ensure that the flow is from outside the containment to inside the containment through any crevices, or other openings in the containment. This air flow then exits the containment through the HEPA filters (Figure 5), through the regenerative blower, out of the building and through a local exhaust blower.

DPT Decontamination Phase—Section “B”: While produce treatments are being remotely executed inside the containment, test materials can be sequentially added to the processing equipment by using the DPT. The DPT and the transfer container are routinely decontaminated independently of the containment by injecting atmospheric steam into the DPT between runs. This ensures that any airborne microorganisms will not escape the containment into the room when the DPT is opened for loading the next produce sample. Produce samples are removed from the containment through the LPT. Produce not needed as samples may remain in the containment through the decontamination cycle.

Containment Heating Phase—Section “C”: Once the experimental trial is complete, the containment is heated by pressurizing the frame with steam and injecting steam from the containment ceiling into the containment atmosphere to achieve a minimum atmospheric temperature of 80°C, as measured at the lower right-hand corner of the containment directly below the DPT (Figure 2).

Dwell Phase—Section “D”: Once the containment temperature reaches 80°C, the program maintains this temperature, or higher, for two hours, which was experimentally determined to be effective for complete inactivation.
of all Escherichia coli ATCC 25922 cells inoculated on the surfaces of the containment and processing equipment within the containment (data not shown). During this time, steam is continuously injected, unmodulated, into the containment, and the blower is continuously operated to maintain the negative pressure. If during the dwell period, atmospheric temperature falls below 80°C, the dwell period would be restarted. Because steam flow is not modulated during the dwell period, the atmospheric temperature continues to rise and typically increases to 90°C or above.

**Containment Cool-down Phase—Section “E”**: Steam flow is stopped, but the blower continues to operate. After fifteen minutes, most of the hot humid air from the containment is removed and the doors may be opened. An additional cooling period of at least 15 minutes is usually desired to avoid potential burns to personnel.

**Validation of BSL-2 Enhanced Containment**

Validation of the BSL-2 enhanced containment was completed under three different conditions using pathogenic *Salmonella* and a non-pathogenic *Escherichia coli*. The validation conditions included containment of aerosol generation and inactivation, decontamination of inoculated stainless steel coupons (SSCs), and validation of a pilot-scale fruit treatment process.

**Bacterial strains, maintenance, growth conditions, and inoculum development**: *Salmonella poona* RM 2350 (California Department of Health Services 00A3563), a clinical isolate associated with a cantaloupe outbreak, was obtained from Dr. William Fett (USDA-ARS-ERRC, Wyndmoor, Pennsylvania, USA). *E. coli* ATCC 25922, a non-pathogenic surrogate for *S. poona* (Eblen et al., 2005), was obtained from American Type Culture Collection (Manassas, Virginia, USA). Stock cultures were stored in tryptic soy broth (TSB; BBL/Difco, Sparks, Maryland, USA) containing 20% glycerol at -80°C. Working stocks were maintained on tryptic soy agar (TSA; BBL/Difco) slants containing 0.6% yeast extract (YE; BBL/Difco) stored at 4°C for two to four weeks. A loop full of culture from a TSA-YE slant was transferred into 10 ml of TSB and allowed to grow for approximately eight hours at 35°C. This culture was then used to inoculate two liters of the same growth medium at 0.01% (v/v) level. The
culture was allowed to grow to stationary phase at 35°C for 18 hours, and was used for inoculation of the BSL-2 containment, SSCs, or cantaloupes. The culture purity of non-pathogenic *E. coli* was assessed using the selective media Cefixime-Tellurite-Sorbitol MacConkey agar (CTS-MAC; BBL/Difco), MAC, and Eosin Methylene Blue agar (EMB; BBL/Difco). The culture purity of *S. poona* was checked using the selective medium Xylose-Lysine-Turgitoll-4 agar (XLT-4; BBL/Difco). Also, cultures were examined serologically for identity, using commercial latex agglutination tests (Ebben et al., 2005).

Validation of aerosol contamination and decontamination of BSL-2 enhanced containment using atmospheric steam treatment: An important function of the BSL-2 enhanced containment is to contain aerosols contaminated with microorganisms, in particular, human pathogens. To validate the containment of aerosols generated inside the BSL-2 enhanced containment, a TSB grown culture of non-pathogenic *E. coli* was sprayed on all reachable surfaces throughout the containment interior using a Hudson Industry pressurized sprayer (H.D. Hudson Manufacturing Company, Chicago, Illinois, USA) with a brass Tee Jet 8004E nozzle tip (Spraying Systems, Co., Wheaton, Illinois, USA). The culture, contained in two 1-liter Pyrex bottles, and the pressurized sprayer were brought into the containment. The two doors to the containment were closed, with the gaskets sealed, and the regenerative blower creating a negative pressure in the containment before spraying. Spraying was done from outside the containment using the glove ports near the LPT (Figure 1) to transfer the culture into the sprayer and to operate the sprayer to create the aerosol. Samples of the culture were taken from the exit of the spray nozzle and tested to ensure viability at the start of each experiment. These, and future samples, were brought out of the containment in Ziploc storage bags through the LPT, as described above, sealed in a leak proof plastic container, and transported to a microbiological laboratory for analysis.

Aerosol testing was completed using a SAS-super100 air sampler (Bioscience International, Rockville, Maryland, USA). Five hundred liters of air were drawn over a TSA plate. During spraying, several air samples were taken outside the containment in areas near the containment doors, near the gaskets joining the two containment modules, and at the regenerative blower outlet. To collect samples inside the containment, the air sampler was then encased in two Ziploc storage bags and passed into the containment through the LPT. Beginning one hour after spraying, air samples were taken inside the containment. After the removal of the sampler, the decontamination cycle was started, incorporating a holding time of two hours, once the atmospheric temperature reached 80°C. After a cool-down phase of 15 minutes, FS Foam Chlor (Zep Manufacturing Product #244424; Atlanta, Georgia, USA) was sprayed along the exterior of the doorway gaskets for added safety before opening the doors of the containment. Air samples were again taken inside and outside of the containment.

In a similar trial, the efficacy of the decontamination cycle to decontaminate surfaces of the containment and its components after being sprayed with *E. coli* suspension, was examined. Before the containment was closed, sample sites were swabbed to ensure that there was no *E. coli* before spraying with that culture. Whirl-Pak® Speci-Sponge® environmental surface sampling bags (NASCO, Fort Atkinson, Wisconsin, USA), hydrated with 25 ml of 0.1% peptone water (FW) (BBL/Difco) were used to swab 8 x 8 cm sections of the window, door gasket, door, ceiling, and parts of the dip tank; a sample was taken of the floor pan water to recover contaminants. The same areas were sampled after the decontamination cycle to recover possible surviving *E. coli*. During spraying, swabs of the door gasket, exterior to the containment, were taken to determine whether *E. coli* had leaked out around the gasket. One hour after spraying with the containment at normal room temperatures, swabs of the interior window surfaces, where the operator could reach using the glove port near the LPT, were taken to confirm that viable *E. coli* contamination was present on the containment’s interior surfaces prior to the decontamination process. All samples were enumerated as described below.

Validation of decontamination of inoculated stainless steel coupons using atmospheric steam treatment: Thirty 304 series SSCs (2 cm x 8 cm x 0.15 cm) with a #4 sanitary finish were inoculated with *E. coli* and *S. poona* in separate trials, by immersion in TSB-grown culture for five minutes, and were allowed to dry for two hours in a biological safety cabinet (BSC) until visibly dry. Inoculated SSCs were stored individually in a 50 ml sterile polypropylene conical tube for 24 hours at room temperature (RT = 19 ± 1°C) prior to treatment. The inoculated coupons were then transported to the BSL-2 facility in a sealed plastic container and were used to validate the process of using atmospheric steam for the decontamination of the BSL-2 enhanced containment. Twenty-seven inoculated SSCs were hung using previously installed hooks in the containment. Nine coupons were mounted by the left door, nine coupons were mounted in the center of the containment and nine coupons were mounted by the right door. The test coupons were equally spaced in the horizontal and vertical planes. After the coupons were mounted, TSB-grown culture of *E. coli* was added to the floor pan and dip tank water, to give a final concentration of 3 log CFU/ml to determine if this water reached a high enough temperature to inactivate the bacteria. Once the test coupons were installed and the water was inoculated, the containment doors were securely closed. To simulate a produce disinfection trial, water in the dip tank was circulated for 15 minutes at the maximum flow rate. At the same time, two air samples were taken near the regenerative blower where the HEPA filtered air was exhausted. TSA plates in the air sampler were each exposed
to 500 liters of this air during the five-minute sampling time. Once the simulated disinfection treatment was completed, the dip tank water and the containment were heated simultaneously to a minimum of 80°C at which time the containment’s two-hour holding time at ≥80°C began, followed by the 15-minute cooling phase. The coupons were then collected and placed into individual sterile 50 ml polypropylene conical tubes containing 30 ml of TSB and sterile glass beads. Water samples were collected from the floor pan and the dip tank. All samples were transported in sealed plastic containers marked with the universal biohazard symbol for microbial analysis, as described below, to a microbiological laboratory. This validation protocol using SSCs was repeated three times.

Validation of pilot-scale process treatment of cantaloupes using the BSL-2 containment: Experiments with cantaloupes were used to validate a pilot-scale thermal surface pasteurization process (Annous et al., 2004) applied in the dip tank inside the containment. Cantaloupes, weighing between 1134 and 1764 grams and free of decay and punctures, were obtained from local suppliers and grocery stores and stored at 4°C for no more than two days prior to use. These cantaloupes were inoculated with E. coli and S. poona in separate trials using the dip method (Annous et al., 2004). Briefly, TSB-grown culture of E. coli or S. poona were centrifuged down at 6740 x g for 20 minutes, washed once with 400 ml sterile deionized water, and suspended in two liters of sterile deionized water to give a final cell concentration of approximately 9 log CFU/ml. This inoculum was used to inoculate cantaloupes as described below. The inoculum was stable in deionized water for at least 24 hours, as seen by the recovery of similar microbial cell densities at time zero, four, and 24 hours (Annous et al., 2004). The cantaloupes were submerged individually in inoculum for five minutes, then drained and allowed to air dry on an absorbent paper for two hours (one hour on each side) in a biosafety cabinet. The cantaloupes were then stored in plastic tubes lined with an absorbent paper at room temperature for 24 hours prior to treatment. The inoculated cantaloupes were then transported to the BSL-2 facility in a sealed plastic container (BMBL, 1999), and loaded into the transfer container in a class II A2 BSC before being introduced into the DPT through which they entered the closed containment and were released manually from outside the containment into the dip tank. By operations of the dip tank, triplicate samples, each consisting of three cantaloupes, were totally immersed in the untreated tap water at RT, or 76°C for three minutes, immediately sealed in plastic bags, and submerged in a water-ice bath for five minutes. Most of the air in the plastic bag was removed prior to sealing to minimize any effect on heat transfer during the cooling process. The cantaloupes were then analyzed for residual surface microbial population using the whole rind method of sampling (Annous et al., 2004).

Between the two temperature treatments, the transfer container and pass-through were decontaminated with steam for five minutes at or above 93°C, and swabs were taken to verify that this operational process decontaminated the DPT. Additional swabs were taken at the DPT door, bottom of the transfer container, the DPT’s internal latching mechanism, and the ramp used to convey the cantaloupes from the DPT to the dip feed. After completion of the cantaloupe decontamination treatments, the containment was decontaminated using the decontamination cycle described above. The dip tank entry and exit ramps were swabbed after the containment decontamination. Water samples from the dip tank and floor pan were also collected and tested for the presence of test microorganism. All samples were enumerated as described below.

Enumeration and Recovery of Bacteria

At the conclusion of trials to validate the performance of the containment (including its use in pilot scale experiments and subsequent decontamination of the system), swab samples, test coupons, water samples, cantaloupes and TSA plates used with air sampling, were removed from the containment area and transported to the microbiology laboratory for analysis in sealed containers as described above. TSA was used for enumeration of total aerobic microorganisms, and as a recovery medium for injured cells of E. coli and S. poona. Enumeration of uninjured E. coli and S. poona populations was done using the selective media MAC and XLT-4, respectively. Recovery medium (TSA) plates were incubated at 35°C for two hours to allow for recovery of injured E. coli and S. poona cells and then overlaid with the appropriate selective medium. All plates were incubated for 24 hours at 35°C, and the resultant colonies were counted manually. Cell densities were reported as log CFU per cm² or per ml.

SSCs: Treated and non-treated SSCs in 50 ml centrifuge tubes containing 30 ml TSB and sterile glass beads were vortexed for one minute. TSB samples were serially diluted and plated on MAC, or XLT-4, and on TSA with a MAC, or XLT-4 overlay. The remaining samples were incubated overnight at 35°C for enrichment and recovery of cells present in numbers below the detection limit, and were plated on MAC or XLT-4. Typical colonies growing on MAC, or XLT-4 were checked for identity using the procedures described above.

Cantaloupes: The whole rind (flesh-free) of a cantaloupe was removed using a sterile Muro Peel-All Fruit Peeler CP-44 (Muro Corporation, Tokyo, Japan), placed in a sterile one-liter glass blender jar (Waring Products, Torrington, Connecticut, USA), combined with four equal volumes (w/v) of PW, and blended at medium speed for one minute with a commercial blender (Waring Blender, Model 51BL31). The resulting blend was filtered through a filter bag (Spiral Biotech, Bethesda, Maryland, USA), and duplicate 10 ml volumes were transferred to sterile tubes. Filtrates were diluted in PW as necessary, and surface plated on the appropriate growth medium as
described above.

Water Samples: Water samples were plated on MAC, or XLT-4 and TSA, with MAC or XLT-4 overlay as described above. Enrichment of water samples was completed by mixing 10 ml of the water sample with 10 ml of sterile double strength TSB, and was incubated at 35°C overnight. Enriched samples were plated on MAC or XLT-4. Typical colonies growing on MAC, or XLT-4 were checked for identity using the procedures described above.

Air Samples: TSA plates used with air sampling were overlaid with MAC, or XLT-4 following incubation at 35°C for two hours as described above. Typical colonies recovered on TSA overlaid with MAC or XLT-4 were checked for identity using the procedures described above.

Swab Samples: Sponges were processed using a Stomacher 400 circulator (Seward, England) at 230 rpm for one minute. The samples were serially diluted and plated on MAC, or XLT-4 and on TSA with a MAC or XLT-4 overlay. The remaining samples were incubated overnight at 35°C for enrichment and recovery of cells present in numbers below the detection limit, and were plated on MAC or XLT-4. Typical colonies growing on MAC, or XLT-4 were checked for identity using the procedures described above.

Scanning Electron Microscope (SEM)

SSCs were examined following inoculation for visualization of attachment and biofilm formation by E. coli and S. poona. Duplicate samples were fixed by immersion in 2.5% glutaraldehyde-0.1 M imidazole buffer solution (pH=7.0) for two hours and stored in sealed tubes at room temperature until further processing. Fixed samples were washed in the buffer, dehydrated in a graded series of ethanol (50%, 80%, then absolute) and critical-point-dried by liquid carbon dioxide. Samples were coated with a thin layer of gold by DC sputtering. Digital images were collected in the secondary electron imaging mode of a SEM Model Quanta 200 (FEI, Hillsboro, Oregon, USA).

Statistical Analysis

Analysis of variance with individual contrasts and Bonferroni T-tests were used to determine significant differences ($p<0.05$) among population means in response to treatments. Unless otherwise indicated, the level of significance used was $p = 0.05$. All statistical analyses and calculations of means and standard deviations were performed using SAS/STAT software (SAS Institute Inc., Cary, North Carolina, USA).

Results and Discussion

The decontamination dwell period was set at 120 minutes, and the data were extracted in 15-minute intervals starting at 45 minutes into the decontamination cycle. The temperatures of the wastewater and atmospheric air at a bottom corner of the containment, and at an additional location directly underneath the DPT (coldest point of the containment), were measured with the instrumentation described above. The temperature of a motor core was measured with a surface mounted thermocouple at the rotor of a stationary 375 W wash down motor (Leeson, Grafton, Wisconsin, USA). The motor was located on the containment floor directly under the DPT next to the RTD. Resulting temperature data are shown in Table 1. Data show that a temperature in excess of 80°C was maintained for over 100 minutes. This time-temperature combination was previously shown to be sufficient for total inactivation of E. coli ATCC 25922 cells applied to surfaces inside the containment and processing equipment placed within the containment system (data not shown).

With the air temperature profile well established, the surface temperature of the internal components was determined for various dwell periods. Twenty-seven test coupons with temperature indicator strips were adhered to the SSCs surfaces. These SSCs were mounted adjacent to the inoculated SSCs. In addition, similar temperature indicators strips were mounted onto high mass surfaces, such as pumps, gear motors, and the floor pans. The read-

<table>
<thead>
<tr>
<th>Temperature (ºC)</th>
<th>0 minutes</th>
<th>45 minutes</th>
<th>60 minutes</th>
<th>75 minutes</th>
<th>90 minutes</th>
<th>105 minutes</th>
<th>120 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Pass-Through (DPT)</td>
<td>98.0</td>
<td>99.3</td>
<td>100.4</td>
<td>100.0</td>
<td>99.2</td>
<td>100.9</td>
<td>99.3</td>
</tr>
<tr>
<td>Wastewater floor (pan)</td>
<td>27.6</td>
<td>76.9</td>
<td>89.6</td>
<td>92.6</td>
<td>92.9</td>
<td>93.1</td>
<td>94.2</td>
</tr>
<tr>
<td>Containment atmosphere (Opposite DPT)</td>
<td>80.4</td>
<td>87.0</td>
<td>87.1</td>
<td>87.4</td>
<td>88.0</td>
<td>87.6</td>
<td>88.6</td>
</tr>
<tr>
<td>Containment atmosphere (Under DPT)</td>
<td>77.1</td>
<td>84.9</td>
<td>85.0</td>
<td>85.4</td>
<td>87.9</td>
<td>86.0</td>
<td>86.0</td>
</tr>
<tr>
<td>375 W (1/2) Hp Motor (Measured at rotor)</td>
<td>58.7</td>
<td>89.6</td>
<td>90.7</td>
<td>91.6</td>
<td>93.7</td>
<td>93.0</td>
<td>93.8</td>
</tr>
</tbody>
</table>
ing of each surface temperature profile was recorded following 60- and 120-minute dwell periods cycle (Table 2). With only a 60-minute dwell period, the highest temperature achieved at the coldest location of the containment was 71°C. Even though this temperature is sufficient to deactivate the bacterial species of concern, the possibility exists of cooler sections inside the containment where these bacterial cells might survive. The goal was to have the containment at a temperature significantly above the kill point of the bacteria, which should guarantee safe entry into the containment. The temperature of containment surfaces were equilibrated at or above 93°C as confirmed by the indicator strips at the end of a 120-minute dwell period.

The surface temperature of DPT was validated by directly attaching indicator strips to the DPT surfaces. One strip was placed inside the transport box; another strip was placed in the mechanism area, and the final strip was placed in the actual pass-through area. Steam was applied to the pass-through for five minutes, while recording the atmospheric temperature profile inside the pass-through. The indicators strips reached their maximum temperature of 93°C, while the atmospheric temperature reached 100°C in two minutes. Air and surface temperature profiles of the containment and DPT following the 120-minute dwell period (Tables 1 and 2) along with the microbiological data, indicated that conditions used for the decontamination of the containment ensured total inactivation of vegetative bacterial cells on the surfaces and in the atmosphere of the containment.

Validation of Aerosol Containment and Decontamination of BSL-2 Containment Using Atmospheric Steam Treatment

TSB grown culture (2 L) of non-pathogenic E. coli ATCC 25922 containing 9 log CFU/ml was sprayed on all reachable surfaces of the containment. Besides contaminating the surfaces, spraying of the culture generated aerosols inside the containment for the purpose of investigating the ability of the containment to contain any potentially airborne microbial cells during the processing of produce inoculated with human pathogens. Cell concentrations of E. coli one hour following spraying on containment and equipment surfaces, and in water on the containment floor, were an average of 7 log CFU per cm² or ml (data not shown). Concentrations of E. coli cells in air samples collected from inside the containment 15 and 60 minutes after spraying, were 3 and 1 log CFU/sample, respectively. All water, surface and air samples (including enrichment) collected from inside and outside the containment prior to spraying were negative for the presence of E. coli. Environmental samples collected from outside the containment during spraying of the culture were negative for E. coli cells, with some exceptions, which are listed in Table 3. In two incidents, a door gasket leaked during spraying. The first incident was due to the lodging of a foreign object between the gasket and the door. The problem was corrected and the Standard Operating Protocols were updated to include inspection and cleaning of these gaskets. In the second incident, the leak was attributed to a rough weld joint at the interface. This weld joint was ground smooth and the remaining joints were inspected and smoothed as necessary. The integrity of the gaskets was re-validated following correction of these problems. Results showed that the gaskets were able to contain the culture and did not allow any leakage to the outside of the containment.

Another potential source of airborne contamination could result from opening the containment door when agent containing aerosols are present inside. It was hypothesized that when the door was opened, a pressure gradient would develop that would transport the bacteria from inside the containment to the exterior work space. To test this theory, investigators sprayed the interior of the containment. After allowing 15 minutes for the aerosol to settle (approximately two containment air ex-

Table 2
Surface temperatures of various locations within the containment following a 60- and 120-minute decontamination cycle as measured by indicator strips.

<table>
<thead>
<tr>
<th>Location</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 minutes</td>
</tr>
<tr>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>Dry Pass-Through End of Containment</td>
<td>71</td>
</tr>
<tr>
<td>Middle of Containment</td>
<td>77</td>
</tr>
<tr>
<td>Liquid Pass-Through End of Containment</td>
<td>82</td>
</tr>
<tr>
<td>Dry Pass-Through</td>
<td>93</td>
</tr>
<tr>
<td>Conveyor Motor</td>
<td>82</td>
</tr>
<tr>
<td>Pump Motor</td>
<td>82</td>
</tr>
<tr>
<td>Floor Pans</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not Determined
changes), the door of the containment was opened and air samples were collected from inside and outside of the containment. These air samples tested positive for *E. coli* (1-3 log CFU/ml). Air samples collected from outside the containment prior to spraying, during spraying, and 24 hours post spraying, were negative for the presence of this microorganism. To validate this design, a TSB grown culture of *E. coli* was added to the water on the floor pan water to give a final cell concentration of 3 log CFU/ml. Water samples collected from the inside of the containment and the outside manifold following the decontamination cycle were negative for the presence of *E. coli* cells. This indicated that following decontamination, the manifold was free of any potential contaminant as a result of processing produce inoculated with human pathogens.

The data presented in Table 3 indicated that the containment unit prevented the airborne contaminants generated during the processing of produce inoculated with human pathogens from entering into the pilot plant facility. Also, atmospheric steam was capable of completely inactivating *E. coli* cells in the air and on the containment surfaces after aerosol generating, or other experiments, without the risk of exposing the operator to pathogens. This allows for opening of the containment for replacing processing lines without the risk of exposing the operator to potential airborne foodborne pathogens.

### Table 3

Recovery of *Escherichia coli* ATCC 25922 cells from air and surface samples collected from containment and equipment following a steam decontamination cycle.

<table>
<thead>
<tr>
<th>Date</th>
<th>Air Window Equip.</th>
<th>Metal Surfaces</th>
<th>Gaskets</th>
<th>Pan Water</th>
<th>Window Equip.</th>
<th>Metal Surfaces</th>
<th>Gaskets</th>
<th>Pan Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feb. 15, 2005</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feb. 1, 2005</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oct. 4, 2004</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sept. 27, 2004</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>May 5, 2004</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>April 21, 2004</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>April 20, 2004</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>April 13, 2004</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feb. 12, 2004</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NG</td>
<td>NG&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> NG = No growth was detected with all samples tested (enriched and non-enriched samples).

<sup>b</sup> Bottom right-hand corner of the seal tested positive for *Escherichia coli* (>4 log CFU/ml) during spraying. No cells were recovered following the decontamination cycle. The problem was attributed to a rough weld joint. This weld joint was ground smooth and the remaining joints were inspected and smoothed as necessary.

<sup>c</sup> Following the decontamination cycle, water samples collected from the pan inside the containment were negative for *Escherichia coli*, but the water collected from the outside manifold was positive for *Escherichia coli* (>4 log CFU/ml). It was determined that the water in the outside manifold did not reach the temperature required to thermal inactivate the bacteria. A heat exchanger tube in the heat exchanger was designed and installed. After the modification, the temperature inside the manifold now approaches 100°C.

<sup>d</sup> Due to a control failure, the containment reached and maintained a temperature of 80°C, or above, for 18 minutes of the 120 minutes. The problem was caused by a programming error, which was later corrected.

<sup>e</sup> A door gasket leak caused bacteria to become airborne. Air samples collected from outside of the containment were positive for *E. coli*. The problem was a foreign object between the gasket and the door frame. The problem was corrected and the Standard Operating Protocols were updated to include inspection and clean-up of these gaskets.

<sup>f</sup> Fifteen minutes following spraying, which allowed 15 minutes for the aerosols to settle, the door of the containment was opened and air samples were collected from inside and outside of the containment. These air samples tested positive for *Escherichia coli* (1-3 log CFU/air sample). Air samples collected from outside the containment prior to spraying, during spraying, and 24 hours post spraying, were negative for the presence of this microorganism.
Validation of the Use of Atmospheric Steam for Inactivation of Bacterial Cells in Biofilm

Decontamination of inoculated SSCs was conducted to validate the ability of atmospheric steam to inactivate microbial cells present in a biofilm. Biofilm formation by *E. coli* ATCC 25922 and *S. poona* RM 2350 on produce surfaces was reported to be responsible for increased resistance to aqueous sanitizing washes (Annous et al., 2004; Annous et al., 2005a; Annous et al., 2005b). Although atmospheric steam inactivated *E. coli* cells on inoculated surfaces of the containment following spraying, cells were not allowed enough time for strong attachment and biofilm formation. Therefore, SSCs were inoculated and dried for 24 hours at RT to allow for strong attachment and biofilm formation on the surfaces. Attachment and biofilm formation by *E. coli* (Figure 7) and *S. poona* (Figure 8) on SSCs’ surfaces were established using SEM. Thirty SSCs, inoculated with *E. coli* or *S. poona* (Table 4), were attached at different locations within the containment and the DPT. All SSCs, including enriched samples, were negative for the presence of *E. coli* or *S. poona* cells following the decontamination cycle of the containment using atmospheric steam (Table 4). These results indicated that atmospheric steam was capable of inactivating cells that might exist in biofilms on the surfaces of the containment and/or equipment within. Also, this would indicate that any microbial contaminants on the surfaces of the containment modules, as a result of processing produce inoculated with human pathogens, would be inactivated by atmospheric steam as previously demonstrated.

Validation of Pilot-Scale Process Treatment of Cantaloupes

Development and fabrication of the pilot-scale dip tank for washing produce or surface pasteurization was completed as part of this research project. Validation of the dip tank for surface pasteurization of cantaloupes inoculated with pathogenic *S. poona* RM 2350, or the non-pathogenic *E. coli* ATCC 25922 within the containment system, was carried out as described above. Surface pasteurization of cantaloupes at 76°C for three minutes resulted in significant reductions in *S. poona* (Table 5) and *E. coli* (Table 6) cell densities compared to the controls. Six of the nine cantaloupes inoculated with *S. poona*, and three of the nine cantaloupes inoculated with *E. coli*, showed no growth on selective and recovery medium.

**Figure 7**
Scanning Electron microscopy image of biofilm formation by *Escherichia coli* ATCC 25922 cells on a stainless steel coupon.

**Figure 8**
Scanning Electron microscopy image of biofilm formation by *Salmonella poona* RM 2350 cells on a stainless steel coupon.

**Table 4**
Efficacy of decontamination process of containment on recovery of *Escherichia coli* ATCC 25922 and *Salmonella poona* RM 2350 cells from inoculated stainless steel coupons.

<table>
<thead>
<tr>
<th>Samples</th>
<th><em>Escherichia coli</em> ATCC 25922 (log CFU/cm²)</th>
<th><em>Salmonella poona</em> RM 2350 (log CFU/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>MAC 4.14 ± 0.53</td>
<td>XLT-4 4.55 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>TS with MAC overlay</td>
<td>TSA with XLT-4 overlay</td>
</tr>
<tr>
<td>Decontamination Treatment</td>
<td>NGb</td>
<td>NG</td>
</tr>
</tbody>
</table>

* Results are reported as the mean ± standard deviation of three independent runs. Each run consisted of three different inoculated SSC.

b NG = No growth was detected with all samples tested including enriched samples.
There was no significant difference between the controls and the washing treatment at room temperature for three minutes (Tables 5 and 6). This indicated that washing with water at room temperature was not able to dislodge, or remove cells of either bacterium attached to the surface of the cantaloupe. This resistance to water wash could be due to attachment of the cells to inaccessible sites on the rind of the cantaloupe and biofilm formation (Annous et al., 2004; Annous et al., 2005a; Annous et al., 2005b; Solomon et al., 2006). These results indicated that both microorganisms were thermally inactivated by this surface pasteurization treatment (Annous et al., 2004; Solomon et al., 2006). There was no significant difference between recoveries of S. poona (Table 5) or E. coli (Table 6) cells on selective and recovery media, indicating that injury was not a factor during surface pasteurization treatments. Therefore, surface pasteurization treatment of cantaloupe can decrease the risk of foodborne illnesses.

Following completion of all processing trials, a 120-minute containment decontamination cycle was completed. The interior surfaces of the containment modules and the DPT, as well as water samples from the dip tank

There was no significant difference between the controls and the washing treatment at room temperature for three minutes (Tables 5 and 6). This indicated that washing with water at room temperature was not able to dislodge, or remove cells of either bacterium attached to the surface of the cantaloupe. This resistance to water wash could be due to attachment of the cells to inaccessible sites on the rind of the cantaloupe and biofilm formation (Annous et al., 2004; Annous et al., 2005a; Annous et al., 2005b; Solomon et al., 2006). These results indicated that both microorganisms were thermally inactivated by this surface pasteurization treatment (Annous et al., 2004; Solomon et al., 2006). There was no significant difference between recoveries of S. poona (Table 5) or E. coli (Table 6) cells on selective and recovery media, indicating that injury was not a factor during surface pasteurization treatments. Therefore, surface pasteurization treatment of cantaloupe can decrease the risk of foodborne illnesses.

Following completion of all processing trials, a 120-minute containment decontamination cycle was completed. The interior surfaces of the containment modules and the DPT, as well as water samples from the dip tank

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Residual population of Salmonella poona RM 2350 (log CFU/cm²)&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XLT-4</td>
<td>TSA with XLT-4 overlay</td>
<td></td>
</tr>
<tr>
<td>2-h control</td>
<td>4.4 ± 0.4 A</td>
<td>5.0 ± 0.4 A</td>
<td></td>
</tr>
<tr>
<td>24-h control</td>
<td>6.0 ± 0.5 AB</td>
<td>6.8 ± 0.4 B</td>
<td></td>
</tr>
<tr>
<td>76°C for 3 minutes</td>
<td>0.8 ± 1.0&lt;sup&gt;c&lt;/sup&gt; C</td>
<td>1.0 ± 1.1&lt;sup&gt;d&lt;/sup&gt; C</td>
<td></td>
</tr>
<tr>
<td>Room temperature wash for 3 minutes</td>
<td>5.7 ± 0.6 AB</td>
<td>6.4 ± 0.6 AB</td>
<td></td>
</tr>
<tr>
<td>Decontamination Treatment</td>
<td>NG</td>
<td>NG</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Salmonella poona populations were enumerated on selective media (XLT-4) and recovery medium (TSA with XLT-4 overlay).

<sup>b</sup> Data are reported as the means ± standard deviations of three independent runs. Each run consisted of three cantaloupes. Means with the same letter are not significantly different (P < 0.05).

<sup>c</sup> Cantaloupes were dip inoculated with Salmonella poona for five minutes, allowed to dry in a biosafety cabinet for two hours, and stored at room temperature for 24 hours prior to the washing treatment at 76°C or room temperature for three minutes.

<sup>d</sup> Although six of the nine cantaloupes tested showed no growth, 0.1 log CFU/cm² rind (minimum detection level) was used to determine the mean and standard deviation.

NG = No growth was detected with all samples tested including enriched samples.

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Residual population of Escherichia coli ATCC 25922 (log CFU/cm²)&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAC</td>
<td>TSA with MAC overlay</td>
<td></td>
</tr>
<tr>
<td>2-h control</td>
<td>5.0 ± 0.4 A</td>
<td>5.1 ± 0.4 A</td>
<td></td>
</tr>
<tr>
<td>24-h control</td>
<td>6.0 ± 0.5 A</td>
<td>6.2 ± 0.5 A</td>
<td></td>
</tr>
<tr>
<td>76°C for 3 minutes</td>
<td>1.4 ± 0.8&lt;sup&gt;c&lt;/sup&gt; B</td>
<td>1.5 ± 0.8&lt;sup&gt;d&lt;/sup&gt; B</td>
<td></td>
</tr>
<tr>
<td>Room temperature wash for 3 minutes</td>
<td>5.4 ± 0.6 A</td>
<td>5.7 ± 0.5 A</td>
<td></td>
</tr>
<tr>
<td>Decontamination Treatment</td>
<td>NG</td>
<td>NG</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Escherichia coli populations were enumerated on selective media (MAC) and recovery medium (TSA with MAC overlay).

<sup>b</sup> Data are reported as the means ± standard deviations of three independent runs. Each run consisted of three cantaloupes. Means with the same letter are not significantly different (P < 0.05).

<sup>c</sup> Cantaloupes were dip inoculated with Escherichia coli for five minutes, allowed to dry in a biosafety cabinet for two hours, and stored at room temperature for 24 hours prior to the washing treatment at 76°C or room temperature for three minutes.

<sup>d</sup> Although three of nine cantaloupes tested showed no growth, 0.1 log CFU/cm² rind (minimum detection level) was used to determine the mean and standard deviation.

NG = No growth was detected with all samples tested including enriched samples.
and pan drains were collected for analysis. All samples, including enriched samples, were negative for the presence of *E. coli* or *S. poona* cells following the decontamination cycle of the containment using atmospheric steam. These results indicate that processing inoculated produce within the containment can be conducted without contaminating surrounding work areas, and that the decontamination of equipment and protocols developed are effective.

**Conclusions**

This manuscript contains descriptions of the design, fabrication and validation of BSL-2 enhanced containment and pilot-scale surface pasteurization treatment of cantaloupes. The validation of a BSL-2 enhanced containment and processing treatment demonstrated that the procedures were fully successful with regards to containing and deactivating airborne, surface attached, and water suspended bacterial cells. The BSL-2 enhanced containment facility offers a unique opportunity to validate antibacterial interventions for decontamination of produce inoculated with human pathogens using pilot and industrial scale equipment. The research community now has a safe, workable, and proven engineering tool providing the ability to conduct pilot-plant scale decontamination studies of produce and equipment using human foodborne pathogens of interest, instead of surrogates.

Surface pasteurization treatment of cantaloupes using hot water meets the USFDA target of 5 log reduction in pathogen levels. The use of hot water as a method to decontaminate cantaloupe is more effective than various other washing and sanitizing treatments tested to date. The work presented in this paper demonstrates the utility of the containment system in validating surface pasteurization as an effective treatment that greatly reduce levels of *Salmonella* on the surface of inoculated cantaloupes.

**Acknowledgements**

The authors acknowledge the technical assistance of Dr. Peter Cooke and Mr. Andrew Bigley at the Eastern Regional Research Center for SEM images and technical services, respectively. Mr. Paul Pierlott, Akima (USDA service contractor) for photographic support. We thank Dr. Gerald Sapers (Emeritus), United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, Pennsylvania, and Joseph Kozlovac, Agency Biosafety Officer, United States Department of Agriculture, Agricultural Research Service, Beltsville, Maryland for their critical review of this manuscript.

**Authors’ Note**

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned. The author for correspondence is Dr. Bassam A. Annous. Dr. Annous can be contacted via telephone at 215-233-6797 or via e-mail at bassam.annous@ars.usda.gov.

**References**


Evaluation of the Public Review Process and Risk Communication at High-Level Biocontainment Laboratories

Margaret S. Race
SETI Institute, Mountain View, California

Abstract

The proposed construction of NIH-NIAID biodefense labs provided an opportunity to study real-time developments in the public review process for high-level biocontainment facilities and compare them with literature findings on other biocontainment labs and complex science/technology projects. The goals of this study were to examine the types of issues and concerns raised in the review process, evaluate the importance of different factors in the public debate, and understand what features, if any, arose in situations with and without controversies. Based on an analysis of environmental impact documents, detailed timelines and media and Internet communications, it was possible to identify common issues and features associated with the lab reviews. Issues of trust and transparency, concerns about secret, or classified research, undisclosed accidents and a lack of due process were repeatedly found in controversial situations. The lessons learned from this study are relevant to current and future biocontainment projects, and highlight the importance of developing trust, implementing comprehensive early proactive risk communication plans, and maintaining open communication even after operations begin. Current governmental restrictions on transparency and openness associated with biodefense and terrorism make communication increasingly difficult, and have implications for long-term public trust and perceptions about biosecurity.

Introduction

In late 2002, the National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIH-NIAID) published a request for proposals to construct multiple biocontainment laboratories in support of its strategic biodefense research plan. This NIAID construction grant program provided an opportunity to study real-time developments in the review, approval and construction of numerous high-level biocontainment facilities and compare them with literature findings about complex science and technology projects in general and other biocontainment labs in particular. The goals of this study were: to identify the types of issues and concerns raised in the review process, to evaluate the importance of different factors in the public debate, and to determine what features, if any, were associated with controversial and non-controversial situations.

Background: Risk Communication and Biocontainment

Research has identified a list of qualitative factors like trust, familiarity, catastrophic potential and individual control that have strong effects on perceptions and levels of concern about risks (NRC, 1989; Slovic, 2000). In addition, there are well-recognized differences in lay vs. expert responses to technological and biological risks that can impact public discussions (Slovic, 2000; Savadori et al., 2004). In essence, how information is shared may be as important as what is presented about a complex project. Those who seek to “educate” people through a one-way delivery of information and insistent assurances about safety are often unsuccessful (Leiss, 1996; Fischhoff et al., 2004).

In the past five years, several researchers have analyzed the siting and construction of some notable biocontainment laboratories, providing case studies of both good and bad community relations experiences (Lofstedt, 2002; Keith & Wagener, 2004; Fell & Bailey, 2005). According to these researchers, lack of communication and miscommunication with the public are major factors in failure, or near failure of projects. While accurate, detailed message content is necessary, it is likewise important to recognize that trust, transparency, competence, and avoiding secrecy are essential for effective risk communication. If the public is distrustful of officials because of credibility problems, past history, or social alienation, even the best-designed risk communication efforts may be unsuccessful, or impeded. Overall, communication must be a proactive dialogue that addresses the needs of diverse audiences and stakeholders, starts from the earliest planning stages of a project, and continues through project operations. In the design of this study, the presence, or absence of all these factors was noted during the experiences at different laboratories.

Previous reviews of biocontainment labs focused mainly on local, or regional environmental impacts; today’s deliberations also include broader public health and biosecurity issues that have arisen in the post 9/11 era. In monitoring the progress of these new NIAID projects, attention was also focused on whether differences between biosafety and biosecurity are reflected in public debates. In this study, biocontainment labs were viewed as test beds for examining an unusual mix of highly emotive issues and activities—combining typical public health
concerns with genetic engineering, bioterrorism, and emerging infectious diseases (EIDs) in one place. Although biocontainment labs represent a technological solution to some vexing societal problems, they also have the potential for accidents, leaks, or diversions that might impact local citizens or the broader public in serious and worrisome ways.

### Research Approach/Methodology

This study is based on information about new and recent BSL-4 and BSL-3 biocontainment facilities with an emphasis on labs in the NIAID biodefense program. Data on facilities were collected from varied sources, including agency data, environmental impact statements (EISs), environmental assessments (EAs), and hearings; web sites, media sources and journal articles; as well as interviews with individuals at NIH, specific facilities/locales, and citizens’ organizations. The main BSL-4 facilities in this study were the Boston National Laboratory (BNL), Galveston National Laboratory (GNL), and the Integrated Research Facility at Rocky Mountain Laboratory (IRFRML). Information was also gathered on three other BSL-4 labs for comparative purposes, including an unsuccessful NIAID proposal from UC Davis, and two Canadian facilities, Winnipeg and Toronto/Etobicoke. In addition, nine NIAID BSL-3 labs were analyzed, as well as one unsuccessful BSL-3 grant proposal from University of Washington, which was included because of its controversial reception locally. In total, the labs represent a mix of successful, unsuccessful and delayed projects.

Most information was collected between 2002 and late 2005, roughly spanning the time from NIAID’s first announcement of the construction grant program to the end of the federal reviews for the labs. Selective monitoring of some labs continued into 2006 in order to follow continuing legal challenges, opposition, or sporadic media coverage and Internet communications. Because the study focused on debate and potential controversy, particular attention was focused on activities of citizens’ groups and relevant opposition concerns.

In general, collected information included data on the project and facilities; accident records; nature of the respective communities and public involvement; risk communication plans and activities; and media and Internet coverage on biodefense, emerging infectious diseases, emergency preparedness, and related topics. The accumulated information was used to compile detailed databases and timetables about most of the proposed NIAID laboratories, and also to determine the impact of local and national events on individual project reviews.

### Findings

The NIAID BSL-4 labs ranged in size from 82,411 to 194,000 gross square feet (GSF) with costs ranging from $67.178 million; the BSL-3 labs ranged from 18,000 to 41,000 GSF, with costs of $7 to 32 million. Direct comparisons between and among the labs were made possible because they were all integral to NIAID’s strategic plan for biodefense preparedness, and required to comply with the National Environmental Policy Act (NEPA). NEPA compliance imposed similar procedures and timetables with public notifications, open release of documents, opportunities for public comment, and formal government response to public input. It also provided information on project alternatives, accident scenarios, and possible mitigation. Each laboratory was also required to outline a risk communication strategy for outreach and public involvement during the review process. NIAID sponsored several workshops for public relations and communication staffs on lessons learned from earlier biocontainment projects, and suggested ways to tailor the information to their individual projects (Hedetneimi, 2005). Summary information on the NIH BSL-4 labs is provided below beginning with the NIAID labs at Galveston, Boston, and Hamilton/RML (Table 1), and followed by comparative information on two Canadian labs and an unsuccessful UC Davis proposed facility.

### Galveston National Laboratory (GNL)

The proposed GNL is in the center of an existing biomedical complex at the University of Texas Medical Branch (UTMB) campus within an 84-acre site with approximately 77 major buildings. UTMB has safely operated high containment laboratories for multiple years, and currently operates a suite of eight BSL-3 labs (over 5,200 sq. ft.) and ABSL-3 labs (2,400 sq. ft.) in the Keller Building, as well the Robert E. Shope BSL-4 lab (2,100 sq. ft.).

The GNL facility was planned to be 82,411 GSF in a new reinforced concrete seven-story building with a range of functional areas, including: BSL-4 and ABSL-4 (Animal) Labs (6,488 sq. ft. and 5,874 sq. ft. respectively); BSL-3 and ABSL-3 labs (8,964 sq. ft. and 8,380 sq. ft. respectively); BSL-3 insectaries (879 sq. ft.); BSL-2 labs (16,368 sq. ft.); and animal support areas (8,733 sq. ft.), office and conference rooms (14,724 sq. ft.), and support facilities (12,001 sq. ft.). The total cost of the lab is estimated at $167 million, with $110 million from NIAID and the remainder matched by UTMB.

The NEPA process for the GNL proceeded in textbook fashion from 2002 to 2005 with essentially no opposition, or public debate about the project. UTMB held numerous community meetings starting two months before the application was submitted to NIAID and continued them for an entire year until the NEPA process began. These community meetings directly followed years of proactive public education about biocontainment associated with an earlier, privately funded UTMB BSL-4 lab, which became operational in July 2004. No lawsuits, or Freedom of Information Act (FOIA) requests were filed against the project, and no accidents or leaks were re-
Table 1
Comparative characteristics of proposed NIAID BSL-4 labs.
NOTE: Highlighted boxes indicate where one lab is distinctly different from the others.

<table>
<thead>
<tr>
<th>Facility and Institution Characteristics</th>
<th>GNL</th>
<th>BNL</th>
<th>RML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross Square Footage</td>
<td>82,411 GSF</td>
<td>194,000 GSF</td>
<td>105,132 GSF (includes upgrades to existing power, boiler, and utility services)</td>
</tr>
<tr>
<td>Combined New BSL-3/4 Area</td>
<td>30,585 sf.</td>
<td>24,000 sf.</td>
<td>9,710 sf.</td>
</tr>
<tr>
<td># New Permanent Jobs</td>
<td>270</td>
<td>660</td>
<td>100</td>
</tr>
<tr>
<td>Facility Cost ($Million)</td>
<td>$167</td>
<td>$178</td>
<td>$67</td>
</tr>
<tr>
<td>Lab Site</td>
<td>Within urban Med./Res. complex in urban area of Galveston, Texas.</td>
<td>Adjacent to Med./Res. complex in highly urban, mixed residential and commercial area of Boston.</td>
<td>Within federal disease research complex adjacent to rural residential area in Hamilton, Montana.</td>
</tr>
<tr>
<td>Recent Accident History (Including During NEPA Review Process)</td>
<td>None</td>
<td>Multiple Tularemia exposures in 2004; Info disclosed by media (1/05).</td>
<td>Lab worker exposures to Q fever (2/05) and Salmonella (6/04); Info released by NIH to media.</td>
</tr>
<tr>
<td>Research History and Expertise</td>
<td>&gt; 20 yrs. expertise in tropical and emerging infectious diseases; safe operating record with BSL-2/3/4 labs</td>
<td>Long-term research expertise, but a new consortium for EIDs; will draw experts from multiple Boston area institutions with strengths in biomed and pharmaceutical research.</td>
<td>100-yr. research history on combating infectious and re-emerging diseases, vector-borne diseases. Existing biocontainment experience</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neighborhood Characteristics and Issues</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Density Nearby</td>
<td>628 persons/sq. mi.</td>
<td>&gt;&gt;16,000/sq. mi.</td>
<td>&lt;20/sq. mi.</td>
</tr>
<tr>
<td>Nearest Residence to New lab</td>
<td>&gt;1000 ft.</td>
<td>~ 300 ft.</td>
<td>400-500 ft.</td>
</tr>
<tr>
<td>% Minority/Low Income Population in Neighborhood</td>
<td>34% / 22%</td>
<td>52% / 48%</td>
<td>4% / 14%</td>
</tr>
<tr>
<td>Env. Justice Concerns Raised?</td>
<td>NO</td>
<td>YES (Civil Rights Action)</td>
<td>NO</td>
</tr>
<tr>
<td>General Tone of Local Comments</td>
<td>Tell us about operations and policies to assure us it will be safe.</td>
<td>Locally Unacceptable Land Use (LULU)</td>
<td>If you must build it, let us know how you’ll do it safely.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Features of Public Debate and Risk Communication</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prominent Scientists on Both Sides of Lab Debate</td>
<td>NO</td>
<td>YES (strong pro/con debate)</td>
<td>NO (scientists mainly pro lab)</td>
</tr>
<tr>
<td># of Active Opposition Groups (Names of Lead Groups)</td>
<td>0</td>
<td>~ 25 (ACE, Safety Net, Conserv. Law Fdn. etc.) see <a href="http://www.ace-ej.org/">www.ace-ej.org/</a> BiolabWeb/Whoelse.html</td>
<td>3 Coalition for a Safe Lab (CSL), Friends of the Bitterroot (FOB), Women’s Voices for the Earth</td>
</tr>
<tr>
<td>In Communication with Other Opposition Groups Elsewhere?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>SDEIS Issued</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>FOIA Requests About Lab</td>
<td>NONE</td>
<td>MULTIPLE</td>
<td>MULTIPLE</td>
</tr>
<tr>
<td># Legal and/or Government Actions During Debate Over Lab</td>
<td>0</td>
<td>&gt; 15 Federal/State/Local</td>
<td>2 (Federal FOIA and NEPA)</td>
</tr>
<tr>
<td># Comments in EIS Process</td>
<td>418</td>
<td>778</td>
<td>366</td>
</tr>
<tr>
<td># Community Meetings in Time Before Proposal Submitted</td>
<td>&gt; 8 in 4 weeks</td>
<td>9 in 3 weeks</td>
<td>0</td>
</tr>
<tr>
<td># Committee Meetings (and Months) Before First EIS Scoping Meeting.</td>
<td>&gt;24 Mtgs. (15 months)</td>
<td>32 Mtgs. (13 months)</td>
<td>~ 10 Meetings (8 months) (only 2 listed in EA)</td>
</tr>
<tr>
<td># Community Meetings in NEPA Process</td>
<td>&gt;&gt;25 (11 mos.)</td>
<td>&gt;150 (22 mos.)</td>
<td>~ 50 (18 mos.) (only 8 listed in EA)</td>
</tr>
<tr>
<td>Public Comments Raised About Nature of Communications</td>
<td>None (Highest EPA rating for EIS: Lack of Objections)</td>
<td>Condescending, arrogant, secretive; misrepresented level of public support; unwilling to meet</td>
<td>Condescending; arrogant; questioned locals knowledge</td>
</tr>
</tbody>
</table>
ported for any other containment labs at UTMB. Ground-breaking occurred with fanfare and media attention in August 2005 accompanied by a scientific symposium on Biodefense and Emerging Infectious Diseases. As of August 2006, UTMB still maintained a comprehensive web site on the lab, which included a construction web cam (www.utmb.edu/gnl/const/index.shtml) to provide updated public information about the status of the project.

Boston National Laboratory (BNL)

The proposed BNL (official name: National EID Laboratories, or NEIDL) at Boston University Medical Center (BUMC) will be part of a new 14-acre research complex with 30 buildings in the south end of Boston, Massachusetts. Owned and operated by BUMC, BNL facilities will enable research collaboration between investigators from BUMC and research institutions and Universities in the Greater Boston area. The BNL will be a new 126-ft. tall building with four stories of occupied biomedical research space and three stories of mechanical/building support. The facility will include BSL-4 (13,100 sq. ft.) and BSL-3 (10,900 sq. ft.) laboratories; BSL-4 and -3 associated animal holding/support space (15,400 sq. ft.); BSL-2 labs (17,700 sq. ft.); clinical research space (3,500 sq. ft.); and office/building support spaces (15,400 sq. ft. and 8,100 sq. ft. respectively). The total cost of the 194,000 GSF facility is estimated at $178 million, with $128 million from NIAID and the remainder matched by BUMC.

The BNL is located in a densely urban environment with a total population of 53,470 people within a 1-mile radius comprised of seven distinct neighborhoods. According to U.S. Census data (2000), densities in the surrounding area average over 16,000 people/sq. mile (from a low of 10,000 to 20,000 people/sq. mile in mixed residential-industrial areas to a high of 67,000 to 105,000 people/sq. mile in residential sections). The nearest residences are located about 300 feet from the BNL site. The population around the lab is approximately 52% minority and 48% low income.

BNL’s NEPA process was more complicated and contentious than GNL’s at every step, including the addition of a supplemental EIS (SEIS) to address the many public concerns raised by the draft EIS (DEIS). Opposition surfaced very early in the planning process and eventually translated into state and federal lawsuits, public protests, and public hearings at the city and state levels, and intense media coverage for months. Midway in the review process, the media disclosed that BUMC had withheld information about lab acquired Tularemia infections that sickened BU workers on two separate occasions in 2004. Public allegations about withheld information resulted in charges of distrust and a renewed scrutiny of the project by public health officials, city and state lawmakers, the media, and opposition groups. BUMC was eventually fined by OSHA for the incident and strongly criticized by the local public health commission. Subsequently, calls for greater oversight of biocontainment labs arose from both the public and elected officials. Throughout it all, BUMC undertook extensive and varied community efforts to educate stakeholders about the lab and seek public acceptance, which included such unusual tactics as full page newspaper ads signed by hundreds of scientists, and paid ads on local transit vehicles. Opposition lawsuits and legal actions at both the state and federal levels hounded the project starting before the NIH award was announced in September 2003 and continuing into late 2006. Even with the start of construction in spring 2006, opponents vowed to continue their legal fight against the lab suggesting that even if the lab is built, it might still be possible to prevent its operation at the intended BSL-4 level.

Rocky Mountain Laboratories Integrated Research Facility (RML-IRF)

The proposed RML-IRF is located in Hamilton, Montana in rural Ravalli County. The RML complex is a state-of-the-art NIH intramural research facility with over 30 buildings on a 33-acre campus. The IRF project will include new high containment laboratory facilities and major infrastructure upgrades, with 105,132 GSF of new space comprised of high containment laboratories, animal research and support space (BSL-4, -3 and -2 labs at 6,760 sq. ft.; 2,950 sq. ft.; and 14,650 sq. ft. respectively) and office/public areas (25,650 sq. ft.); and infrastructure improvements including a boiler addition (1,810 sq. ft.) and chiller/mechanical area (51,288 sq. ft.). Project costs are estimated at $67 million, supported by NIAID intramural funds. The area around RML has a population density of only 20/sq. mi., with less than 4% minority and 14% low income residents. Overall, the EIS reported that the project would have only slight impacts on the traffic, water and sewage infrastructure. Positive local economic effects were anticipated from construction and operation of the project ($18.9 million during construction and 100 new lab employees upon completion).

The NEPA process for the RML-IRF was somewhat contentious from the start, although less so than in Boston. Problems began with the announcement of a planned Environmental Assessment (EA) process for the proposed BSL-4 lab in February 2002. There were no community meetings prior to the first EA scoping session in July 2002 during which citizens questioned why a comprehensive EIS was not being done. A new citizens group (Committee for a Safe Lab) was established in August 2002 and a coalition of citizens’ groups held public meetings to discuss the project and argue that a full EIS should be conducted. Within a month, NIH announced that an EIS would be completed and the NEPA process resumed with a revised schedule.

Many questions about the project remained after the DEIS was released in May 2003, leading to another formal FOIA request by citizens’ groups in August 2003 and...
a subsequent SDEIS, which was released in late December 2003. Near the end of the NEPA process, RML added a public affairs staff member to keep the public updated on RML activities (October 2003). In spring 2004, the coalition of citizens’ groups filed a FOIA lawsuit to obtain information it had previously requested. Upon completion of the NEPA process, the citizens’ coalition asked for a meeting with the RML Director in August 2004; when their request was denied, the coalition filed a NEPA lawsuit requesting the EIS be redone and a temporary restraining order (TRO) be issued against the lab (Kaiser, 2004a). Within a week a federal judge dismissed the TRO, but ruled that the NIH and citizens’ groups must engage in mediation to resolve the issues (Kaiser, 2004b). A final resolution was reached through mediation in late September 2004 (U.S. District Court, 2004).

Construction of the lab was 95% complete as of August 2006. Local and state political ramifications from the lab were minimal throughout the public debate. Once a formal agreement was reached, communications between the lab and the community improved considerably due, in part, to seminars and facility tours, the disclosure of draft emergency plans, increased citizen involvement on RML committees, and the development of a new master plan (Race, 2006).

Overview and Comparison of the Decision-Making Process at GNL, BNL, and RML Laboratories

The collective experiences at these three BSL-4 labs were quite different: Boston’s was the most controversial; RML’s was moderately contentious; and Galveston’s was essentially routine and trouble-free. An analysis was undertaken to determine if specifics about the labs would correspond in any ways with the observed order of difficulty. Would the similarity or dissimilarity of important features such as the physical nature of the individual projects; their local areas; or the nature of the public debate presage the respective controversies, or lack thereof? Table 1 presents comparative characteristics of the three NIAID BSL-4 labs, with highlighted boxes indicating situations where one project appears distinctly different from the other two.

Institutional and Facility Characteristics: The Boston lab clearly had the most contentious review and was also different in a number of institutional and facility characteristics. BNL is the largest project in GSF, the most expensive ($178 million vs. $167 and $67 million for GNL and RML respectively); and will create more than double the number of permanent jobs as the other labs (660 vs. 270/200 for GNL and RML). All three labs are similarly located within, or adjacent to established medical/research complexes, and will continue biomedical uses on the proposed sites. GNL was alone in having no lab accidents during the NEPA process. BUMC experienced the highly publicized Tularemia infections during the public review process that were disclosed by the media via an anonymous tip. The poor reporting of the Tularemia infections ultimately led to the dismissal of BUMC’s Chief of Infectious Diseases, the very person who was responsible for biosafety training in the new BNL. Although lab accidents also occurred at RML, they were disclosed first by NIH rather than by a news report and didn’t translate into a public issue in the lab debate. Finally, BUMC was different from GNL and RML in its research history and expertise. While BUMC and collaborating institutions are well-known for their strong biomedical and pharmaceutical expertise, they do not have the same publicly acknowledged history of biocontainment research on highly infectious animal, tropical, or EID’s as RML and UTMB.

Neighborhood Characteristics: The neighborhood characteristics and issues for BNL were different from RML and GNL in every feature. The BNL has by far the highest population density, orders of magnitude greater than the others (>16,000 vs. 628 [GNL] and <20 person/mi2 [RML]). BNL was also located closest to nearby multi-family residences (300 feet for BNL compared with >1,000 feet and 400-500 feet for GNL and RML respectively). Equally as important, significant focus was placed on environmental justice and socioeconomic issues for the BNL, but far less at the other two labs (BNL had a 54% minority/48% low income population vs. 34%/22% and 4%/14% respectively at GNL and RML). In Boston, this difference was associated with subsequent civil rights action against BNL, but not at the other labs. Finally, the local citizens’ groups in Boston maintained their opposition from the start based on their premise that the lab project was an example of locally unacceptable land use in a highly urban area (LULU); whereas both GNL and RML public comments focused on mainly operational issues associated with known facilities (essentially a focus on safety).

Public Debate and Communication: Analysis of the communication features also revealed important differences. In Boston, hundreds of prominent scientists were involved on both sides of the debate about the lab, whereas scientists at RML and GNL were mainly probab if involved at all. Boston had the largest number (over 25) of highly organized environmental and citizens’ groups involved, although thee groups led the main opposition. RML had fewer opposition groups in the area, but the three active groups were very knowledgeable about the NEPA process and willing to challenge the project legally, similar to opposition groups in Boston. Both the Boston and RML opposition groups availed themselves of FOIA requests and lawsuits, and were active on many fronts including NEPA process, online newsletters, media releases, protests, ad campaigns, legal actions, and fund raising. RML and BNL opponents also communicated with citizens’ groups elsewhere who were fighting local labs and/or pushing for greater oversight of biodefense research.
nationwide. In Galveston, no citizens’ groups opposed the GNL facility, or were involved in the nationwide debate over biodefense research, even though there are groups in the area that have challenged other projects related to oil drilling, and transportation plans (Curtis, 2006).

GNL and Boston were similar in the ways they handled initial community meetings (eight to nine small group meetings in the month before the lab announcement), while RML had no separate community meetings prior to the lab announcement since it was a congressionally mandated project, and began with less lead time than other BSL-4 labs (Race, 2006). Of the three NIAID labs, GNL had the longest duration risk communication program in advance of the new lab announcement, dovetailing with outreach on biocontainment that began in 1997 for UTMB’s BSL-4 Shope Lab. Both Boston and GNL held a string of community briefings leading up to the first NEPA scoping meeting (24 and 32 meetings in one year). Data supplied by RML staff, but not reported in the EA, indicates that there were about 10 public meetings held during the eight months leading up to the first EIS scoping session and 50 community and local presentations during the NEPA process (Race, 2006). Surprisingly, the lab with the greatest controversy had mounted the largest number of community meetings—more than 150 meetings during the NEPA process for BNL compared with 25 and 50 for GNL and RML.

Finally, while GNL received no negative comments about its communications and EIS process, interviews and media accounts for both RML and BNL indicated that citizen opponents were upset over the tone of their meetings and interactions with experts (Race, 2006). They felt that the experts were dismissive of their questions, considered their concerns baseless, and were telling them what would be done rather than discussing public concerns; moreover, individuals expressed offense at being talked down to by “arrogant, condescending experts.” In both locations, requests for more information about the planned labs were denied, leading eventually to opponents’ FOIA requests and later lawsuits to obtain details.

Comparison of Public Concerns about NIAID BSL-4 Labs

Further analysis was undertaken to discern any patterns in public concerns that might correspond with the degree of controversy at each location. Table 2 indicates the types of public concerns raised in the NEPA process for the three BSL-4 Labs. An attempt was made to review public comment and attitudes associated with the first BSL-4 Lab in Galveston (Shope Lab) to determine if they mirrored the pattern seen with GNL, particularly since the literature indicated that early meetings were hostile. The Shope Lab was planned to address UTMB’s mission in public health and EIDs, while the new GNL focuses on EIDs as well as biodefense and bioterrorism. However, no EIS was done for the Shope Lab because it was privately funded and there is no Texas equivalent of a NEPA-like review process, making comparison impossible (Curtis, 2006). The analysis was compiled using reported comments from the final EISs as raw data with numbers indicating the combined total of oral and written comments submitted by category during the entire NEPA process for each lab. To understand more about these differences, information in Table 2 was further analyzed by focusing preferentially on all categories that encompassed the top five categories for each lab (highlighted in Table 2). Because the original NEPA comments fell roughly into 15-20 major categories, any category representing over 10% of the comments was deemed noteworthy.

Figure 1 is a graphic presentation of these top NEPA comment categories showing the strong variation in local responses. In the categories related to Normal Operations, all three labs recorded many questions, although the emphases were different among them. In general, the GNL comments emphasized questions on what operations and policies would be implemented. This is consistent with a citizenry that is familiar with this type of lab, because one already exists in their area. For RML, the main focus was on emergency response plans rather than normal operation per se, emphasizing the need to know about emergency coordination with local first responders and public health officials. Like RML, Boston’s questions also emphasized Emergency Response Plans and evacuations, rather than normal operations, and were particularly focused on the difficulty of evacuation and emergency response to a biological crisis in densely populated Boston. Repeatedly they asserted that a BNL in the planned area was a locally unacceptable land use (LULU). Interestingly, in Galveston, few questions were asked specifically about emergency response plans, perhaps because of trust, or firsthand experiences (the successful shutdown of UTMB’s labs according to written plans in the face of approaching hurricanes Katrina and Rita in 2005).

Citizens in Boston asked very few questions related to local environmental impacts of various types, perhaps because the site is located in a highly urban area and citizens were focused from the start on opposition, not operations. In contrast, both GNL and RML queries focused on how emissions, wastes, or infrastructure demands might impact their local area. Citizens near RML were particularly concerned about waste and pollution (mainly air pollution via incineration) that might impact the valley.

For BNL and RML, but not GNL, comments in the category of NEPA requirements emphasized the lack of “alternatives” presented in the EIS. The well-organized opposition groups in both Hamilton and Boston were familiar with NEPA compliance expectations, which typically involve the analysis and discussion of at least several alternative sites in detail. In all three EISs, NIH analyzed only the proposed site and the no action alternative, dismissing all other potential sites because they didn’t meet the needs of the NIH program. In Galveston, this lack of
Table 2
Comparison of Public Comments Raised in NEPA Process. Indicates actual numbers and percents of total comments received in public meetings, scoping sessions, phone calls, e-mails and/or comments in response to DEIS and SDEIS documents. NOTE: Bold/highlighted numbers indicate top five categories of concern for each lab.

<table>
<thead>
<tr>
<th>Comments by Category</th>
<th>GNL</th>
<th>GNL</th>
<th>BNL</th>
<th>BNL</th>
<th>RML</th>
<th>RML</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>#</td>
<td>%</td>
<td>#</td>
<td>%</td>
<td>#</td>
<td>%</td>
</tr>
<tr>
<td><strong>Normal Operations and Plans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health and Human Safety</td>
<td>41</td>
<td>9.8%</td>
<td>44</td>
<td>5.7%</td>
<td>6</td>
<td>1.6%</td>
</tr>
<tr>
<td>Policy/Operations</td>
<td>58</td>
<td>13.9%</td>
<td>0</td>
<td>0.0%</td>
<td>8</td>
<td>2.2%</td>
</tr>
<tr>
<td>Emergency Response Plans</td>
<td>6</td>
<td>1.4%</td>
<td>100</td>
<td>12.9%</td>
<td>39</td>
<td>10.7%</td>
</tr>
<tr>
<td>Classified Research/DNA Research</td>
<td>0</td>
<td>0.0%</td>
<td>19</td>
<td>2.4%</td>
<td>12</td>
<td>3.3%</td>
</tr>
<tr>
<td><strong>Local Impacts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infrastructure Utilities</td>
<td>26</td>
<td>6.2%</td>
<td>0</td>
<td>0.0%</td>
<td>22</td>
<td>6.0%</td>
</tr>
<tr>
<td>Waste Management Pollution</td>
<td>36</td>
<td>8.6%</td>
<td>16</td>
<td>2.1%</td>
<td>64</td>
<td>17.5%</td>
</tr>
<tr>
<td>Traffic and Transportation</td>
<td>20</td>
<td>4.8%</td>
<td>4</td>
<td>0.5%</td>
<td>8</td>
<td>2.2%</td>
</tr>
<tr>
<td><strong>NEPA Required Information</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternatives</td>
<td>14</td>
<td>3.3%</td>
<td>87</td>
<td>11.2%</td>
<td>43</td>
<td>11.7%</td>
</tr>
<tr>
<td>Socioeconomic and Env. Justice</td>
<td>34</td>
<td>8.1%</td>
<td>106</td>
<td>13.6%</td>
<td>15</td>
<td>4.1%</td>
</tr>
<tr>
<td>Risk Assessments, Scenarios, Terrorism</td>
<td>97</td>
<td>23.2%</td>
<td>200</td>
<td>25.7%</td>
<td>70</td>
<td>19.1%</td>
</tr>
<tr>
<td>Proposed Action</td>
<td>26</td>
<td>6.2%</td>
<td>0</td>
<td>0.0%</td>
<td>3</td>
<td>0.8%</td>
</tr>
<tr>
<td>Cumulative Impacts</td>
<td>5</td>
<td>1.2%</td>
<td>15</td>
<td>1.9%</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Procedural Matters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Due Process, NEPA Compliance, Access to Information, Trust</td>
<td>19</td>
<td>4.5%</td>
<td>161</td>
<td>20.7%</td>
<td>59</td>
<td>16.1%</td>
</tr>
<tr>
<td>Misc. (Costs, Schedule, Outside Scope, etc.)</td>
<td>36</td>
<td>8.6%</td>
<td>26</td>
<td>3.3%</td>
<td>17</td>
<td>4.6%</td>
</tr>
<tr>
<td><strong>Total # Comments</strong></td>
<td>418</td>
<td>100.0%</td>
<td>778</td>
<td>100.0%</td>
<td>366</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 1
Comparison of comments for NIAID BSL4 Labs.
analysis of multiple alternative sites never became an issue, perhaps because UTMB had communicated to the local public for at least a decade about plans to build BSL-4 facilities and become a world class research center on tropical and emerging infectious diseases.

Socioeconomic and environmental justice concerns were absent at RML, but evident at both GNL and Boston, but for different reasons. Opponents in Boston argued strongly that the large proportion of minority and low-income people living near the proposed lab translated to serious environmental justice concerns. They filed a formal complaint under the Civil Rights Act with the federal Department of Health and Human Services (HHS) asserting that the project would disproportionately impact disadvantaged groups, a position in contrast to the assertions in the EIS. (A decision on this federal civil rights action was still pending in late 2006.) In Galveston, the socioeconomic concerns focused not on minority, or low-income residents near the lab, but rather on the equitable distribution of benefits from new jobs, particularly during construction.

All locales used the NEPA process similarly when asking about their projects’ risk assessments, worst case scenarios, and terrorism risks. Citizens wanted to know how NIH reached its conclusions that construction and operation of the labs would not result in accidental exposures, whether by system malfunction, human error, or terrorist acts. Clearly, citizens have come to expect that the environmental review process—whether federal or state—will present important information and fully address their concerns.

Dissatisfaction with responses, concerns about withheld information, questions about trustworthiness, and repeated comments about lack of due process were recorded at both Boston and Hamilton, but not at Galveston. They were also accompanied by charges that the EIS analyses were inadequate, or improperly conducted, leading to multiple lawsuits of several types (NEPA, MEPA, and FOIA). The GNL, on the other hand, received very few questions related to legal compliance, and essentially none suggesting any concerns about withheld information, or mistrust. For GNL, risk communication efforts throughout the NEPA process, and continuing through construction via web site, web cam, or public symposium on EIDs appear to have reassured the public that they will be kept adequately informed about the lab and its activities as needed.

For the RML project, concerns about due process, secrecy and emergency planning were resolved through court action and legally binding mediation (Kaiser, 2004a and 2004b), which ultimately allowed the lab to be built, and also established a new openness and involvement with the community. The resolution of issues at RML suggests that it is possible to regain trust and move forward, but it takes proactive communication with the stakeholders (Keith & Wagener, 2004). While the NEPA and FOIA lawsuits brought by a coalition of local citizens’ groups were unwelcome challenges to RML administrators, the mediated outcome provided a sense of closure and ushered in a new beginning and a welcome dialogue of sorts. Based on interviews with key members of the citizens’ groups and a lab staff member (Race, 2006), RML is committed to a risk communication plan and has been holding regular seminars and lectures for the public featuring researchers from the lab. RML officials also agreed to provide information on emergency plans and notifications, to increase the number of community members on the Institutional Biosafety Committee, to move the meetings of the Community Liaison Committee to more accessible locations, and even to phase out a long-contentious incinerator for general refuse disposal. As indication of the improved relations, citizens’ opposition and protests ceased around the time that construction of the new lab began.

In Boston, it is premature to say how the multiple legal challenges over the BNL proposal will eventually be resolved, although the citizens’ groups vowed to continue efforts to stop the lab from being built even after construction got underway in May 2006. Challenges in courts and regulatory arenas continue at the time of this writing in September 2006 including New lab oversight regulations by Boston Public Health Commission; a state court ruling and an agency requirement that BU submit a revised supplemental MEPA environmental impact report; and a citizens’ legal request for a TRO against NIH to stop funding of the BNL until federal NEPA challenges are resolved.

**Issues Repeatedly Observed in Public Reviews of High Containment Biolabs**

Analysis and monitoring were also undertaken on 13 other biocontainment labs, although in less detail than the three discussed above. Information was gathered from published reports on an unfunded BSL-4 lab proposal from UC Davis, and two previously built Canadian BSL-4 labs (Fell & Bailey, 2005; Lofstedt, 2002; Keith & Wagener, 2004; Enserink, 2000a and 2000b). Ten BSL-3 labs were also analyzed using a combination of NEPA documents, media reports and information from web sites. None of the nine funded NIAID BSL-3 projects resulted in much attention, or debate in community meetings, or generated any public controversies. Even two projects with accidental escape and loss of lab animals during the review process (New Jersey, Tulane) did not experience increased concern or opposition over the lapses. The only controversial BSL-3 lab was one proposed by the University of Washington, which experienced intense local opposition due primarily to concerns about secrecy, classified research and inappropriate site location. (Details on the UW lab controversy and public concerns are available from the author at mracemom@aol.com.) The combined information from all 16 labs was further analyzed to com-
Previous researchers have suggested that the effectiveness of risk communication is associated with certain key factors, most importantly trust, transparency, and two-way information exchange (Slovic, 2000). Table 3 was compiled using a combination of NEPA comments and an extensive review of media and Internet information on each lab studied. Using data from this study and other published reports, the table records the presence, or absence of, key issues in projects with and without controversies. “Presence” of issues is based on whether a particular issue was specifically and repeatedly documented during the public discussions of all types, not just in NEPA comments. The table also indicates the status of each facility, noting whether it is completed, or largely built, delayed, operational, or stopped completely as of August 2006. Figure 2 provides a graphical presentation of the same information.

Of all the labs studied, the Galveston Lab was the only major facility that was entirely free of controversy, a fact attributed at least in part to its long-term, open and proactive communications (Curtis, 2006). As a group, the smaller, less costly NIAID BSL-3 labs were likewise uncontroversial, perhaps in part because of the widespread occurrence and familiarity with BSL-3 labs in general. The five other BSL-4 labs in this study and the controversial UW BSL-3 lab each experienced varied problems with repeated public, legal, or political challenges and accompanying delays of various lengths. The outcome for the BNL is still awaiting legal rulings (September 2006) that will determine whether it can be completed, or operated.

Focusing specifically on those labs where the controversies occurred, serious and persistent questions of trust were evident in every case, whether briefly, or for a sustained time. Even Winnipeg with its exemplary, proactive, and long-term communications strategy admitted that a minor incident involving undisclosed release of wastewater into the sewer (reported by the media), damaged their reputation, caused a long delay and required a concerted effort and additional time to rebuild the public trust (Keith & Wagener, 2004). The evidence for trust as a top priority in public decision-making about biocontainment labs reaffirms the findings and assertions of many previous researchers. Attention to factual details is clearly essential in reviews of complex science/technology projects, but without trust this information can become secondary in the debate.

Strong concerns about secrecy and classified research were evident in two-thirds of labs with controversies. Only the two Canadian BSL-4 labs had no concerns raised about secret or classified research. This highlights a difference between the purely public health missions of the past and the current blurring of public health, biodefense and bioterrorism in the post-9/11 era. The issue of classified research and secrecy was also linked to citizens’ concerns about emergency responses and restrictions on notifications about what select agents are being used. University-associated labs experienced especially heated debates over secrecy and classified research, which could impact the open publication of research re-

### Table 3

<table>
<thead>
<tr>
<th>LABS: GNL</th>
<th>LABS: BNL</th>
<th>LABS: RML</th>
<th>LABS: UCD</th>
<th>LABS: Toronto</th>
<th>LABS: Winpg</th>
<th>NIAID BSL-3s</th>
<th>UW BSL-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISSUES:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trust</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Classified Res.</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Due Process</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Transparency</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Actual Accidents</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Res. Agenda</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Location (LULU)</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pollution</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

**Labs**: Darkened boxes indicate labs where controversies occurred.

**Status**: B = Built or currently under construction; O = Operational; S = Stopped; D = Delayed; * = details not studied

**Issues**: Highlighted boxes (X) indicate specific issues documented in NEPA documents and/or media coverage at labs where controversies were analyzed.

** = two accidents occurred at NIAID-BSL-3 labs, but each was disclosed by NIH rather than the mass media and resulted in no controversy.
sults. The mixed messages and flip-flopping of interpretations on how the Bioterrorism Act would apply to future research at NIAID biodefense labs no doubt contributed to this continuing concern.

Due process was also an issue in two-thirds of controversial labs. Controversies over both U.S. and Canadian labs demonstrate that serious challenges can, and will, be mounted using available regulatory, court, media, and political avenues even if the opposition represents only a minority of the public. When institutions decide how to fulfill the review process (EA rather than EIS, or a limited number of alternatives analyzed) they may set the stage for potentially greater conflict by providing legal hooks for court challenges by opposition groups. Given the controversial nature of biodefense labs, and the anxiety over possible accidents and terrorism, it is not surprising that due process issues were used as impediments to these projects.

Half the controversial labs shared four additional issues in common: a lack of transparency, accidents, questions about local control of research agenda, allegations about locally unacceptable land uses (LULU as distinct from NIMBY (not in my backyard). Transparency in this situation refers to the open release of relevant information needed to evaluate a project during its review and also relates to institutional attitudes and actions regarding the release of information in general. Repeatedly, when information was withheld, dialogue began poorly and rarely improved without extensive work. The lack of transparency is not unique to this era of biosecurity and secrecy; it was also one of the issues responsible for the difficulties at the Etobicoke (Canada) lab a decade ago. At every turn, a lack of transparency impacted the sense of trust. Interestingly, neither UW nor UCD labs registered concerns about transparency as each of them provided extensive and open information from the start. Thus, transparency is a necessary starting expectation, but other issues may have greater weight in the debate overall.

Accidents were noted in a number of labs, but concern and controversies over accidents become notable only in situations with non-disclosure of information by the sponsoring institution. In essence, it appears there was more concern about institutional trust than the accident itself. This is consistent with recent experimental research on increases and decreases of public trust (White & Eiser, 2006). How risk managers respond to events may be interpreted as indicative of competence, or lack thereof, as well a reflection of institutional attitudes toward the public.

Issues over local control of research agendas were evident mainly for University facilities. Both faculty members and the public raised questions about the required commitment of 20 years for an NIAID research mission, particularly in light of changing legal interpretations related to terrorism, federal government authority, and citizens’ rights. Reassurances from NIH about local control of research agendas, and restrictions against classified research apparently did not satisfy opponents.

Finally, while a number of individuals probably have NIMBY attitudes about any projects in their areas, opposition groups in the recent lab controversies acknowledged the importance of constructing biocontainment labs, but presented strong reasons why particular labs were inappropriate for selected locations, or whether too many were being built nationwide. Whether the LULU issues eventually prevail will depend on judgments of the courts, or perhaps political considerations (Toronto/Etobicoke lab). Nevertheless, both site appropriateness and consideration of alternative sites remain important elements in environmental decision-making. Surprisingly, concerns about local pollution and related impacts occurred in only one-third of the lab controversies.
Conclusion

The findings in this study are consistent with earlier research on risk communication, highlighting the importance of trust and open communication in public decision-making, particularly when it comes to biocontainment labs. Even if a project has political and majority support, it is clear that the activities of a small number of opponents can significantly impede a project, or even cause its demise through legal challenges and other actions. Lack of trust was manifest at every lab where controversies occurred, leading to varying combinations of legal or political challenges, construction delays, or even project cancellation. Concerns about secrecy/classified research and a lack of due process were also seen in two thirds of controversial labs. Lack of transparency, undisclosed accidents, concerns about local control of research agendas, and assertions of unsuitable site locations were observed in half of all controversial labs. Unlike BSL-4 labs established prior to 9/11 when public health, emerging diseases, and biosafety predominated the discussions, the current crop of biodefense labs experienced the added burdens of biosecurity and bioterrorism concerns, as well as questions about secrecy and research control, particularly for university-associated facilities. Interestingly, of the 10 BSL-3 labs studied, only one experienced intense local debate, which coincided with persistent questions about trust, secrecy, research control, and unsuitable location, similar to the features of controversial BSL-4 labs.

Biosecurity related issues (as opposed to biosafety per se) have clearly complicated the current debate over current planned BSL-4 labs for NIAID. Since trust, transparency and information availability are at the very center of effective risk communication, anything interfering with the flow of needed information is likely to be problematic. The current lack of openness related to biosecurity and biodefense information likely contributed to undermining trust in risk managers and officials associated with the labs. At this juncture, finding ways to rebuild trust and demonstrate suitable transparency and openness with the public will be important when labs become operational. Recent research has shown that building, or losing trust is associated with how honest and open the risk managers are perceived to be by the public, and how they handle accidents, or complex events in the face of uncertainty (White & Eiser, 2006). The challenge of finding a balance between public security and public openness will continue to be particularly difficult in the face of current concerns about terrorism and the continuing debate over biodefense priorities and spending.

Although this cohort of NIAID biocontainment labs was required to have risk communication plans as part of their project plans (Hedetneimi, 2006), it is likely they were developed for local siting and decision-making considerations and drew from lessons learned in earlier biosafety and public health debates. Because the communication plans were crafted before biodefense-associated issues were widely debated, they may have omitted the important underlying fact that the BSL-4 biodefense/biocontainment labs have come to represent a highly dreaded risk, in essence a stigmatized technology (Flynn et al., 2001). Collectively they now exhibit the same mix of negative emotions, risk perceptions and stigma associated with other dreaded risks like “nuclear” or terrorism (Peters et al., 2004; Gigerenzer, 2006). As such, they represent low probability, high consequence risks that can cause both direct damage (in this case from accidents with infectious agents) and indirect damage, mediated through the minds of citizens (Lightstone et al., 2006). It is known that people tend to react to dreaded risks with avoidance behavior (Slovic, 1987). Even in situations involving terrorism and the voluntary risks of travel, worry and emotion have been shown to play significant roles in judgments and decision-making (Fischhoff et al., 2004).

For the proposed BSL-4 biocontainment labs, the exposure to hazards associated from biodefense research is entirely involuntary, and leads the public to focus heavily on a host of novel uncontrollable risks, both perceived and actual. In addition to concerns about terrorism, biocontainment labs also involve recombinant DNA technologies and genetic engineering, which are also perceived differently by experts vs. the public (Savadori et al., 2004). For those who conduct research on hazardous biologic agents, the labs and associated risks are familiar, understood and accepted; such is not the case with lay audiences. In addition, experts judge the risks of medical biotechnology and its applications as less harmful, more useful, and more familiar than lay audiences, who focus far more on potential harm. In the current debate over biocontainment labs, the experts are emphasizing societal benefits from conducting needed research, while the concerned public is focusing on personal and local risks, or the broader political dimensions of biodefense. This technological stigmatization has become a powerful aspect of the current debate—one that has not yet been addressed adequately during public communications. It is clear that attempts to educate the public about real vs. perceived hazards of biocontainment and biosecurity involve far more than just the dissemination of scientific and technological information. Future risk communication plans about biodefense research and biocontainment labs will need to incorporate an understanding and acknowledgement of how strongly emotional and affective reactions can impact behaviors and skew public responses. In addition, it will be important that institutions address lingering concerns about trust, lab oversight and operations once labs are permitted to go “hot.” If risk communication is confined mainly to the approval and construction phases of a project, it is not likely to be sufficient. Ongoing communications involving true public dialogue and engagement—not just press releases and announcements—must be part of the lifetime of these facilities. More than
ever, effective risk communication is essential for maintaining and, in many cases, rebuilding public trust both locally and nationally in this current era of bioterrorism and biodefense concerns.

Acknowledgements

This study was begun at the Center for International Security and Cooperation (CISAC) at Stanford University during a research fellowship supported by Carnegie Corporation of New York and the John D. and the Catherine T. MacArthur Foundation. Subsequent work was partially funded by the Jet Propulsion Laboratory, California Institute of Technology, under contract to NASA.

References


Race, M. S. (2006). Personal communications. Many interviewees during this study (2004-2006) requested their names be withheld from being published. Staff and individuals from the following institutions/groups provided important facts and interpretations: BU, RML, UTMB, NIH-Bethesda, ACE, Safety Net, Citizens for a Safe Lab, Friends of the Bitterroot, Women’s Voices for the Earth, Tri-Valley CARES, Canadian Health Agency, Sunshine Project, and selected BSL-3 lab sites.


Biosafety Tips
Karen B. Byers
Dana-Farber Cancer Institute, Boston, Massachusetts

Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem, or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column, I share biosafety insights for managing a variety of workplace situations. I welcome feedback and suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

Cell Sorters Present Containment Challenges
The risk assessment for cell sorting is even more challenging with new high-speed cell sorters and “user friendly” cell sorters appearing in BSL-2 and BSL-3 laboratories, where the users may not be as experienced as flow cytometry technicians in dedicated facilities. Fortunately, the International Society for Analytical Cytometry (ISAC) has published new biosafety guidelines for unfixed cells that are run on a jet-in-air flow cytometer or a cell sorter that combines a flow cell with jet-in-air sorting (Schmid et al., 2007). ISAC provides this comprehensive biosafety reference on its web site at www.isac-net.org/committees/biosafety.htm. The following URL contains the document “Standard Safety Practices for Sorting of Unfixed Cells” www.mrw.interscience.wiley.com/emrw/9780471142959/cp/cpcy/article/cy0306/current/pdf. This version replaces the 1994 ISAC biosafety guidelines and addresses the increased risk of some instruments as well as the improved aerosol control methods available for live cell sorting. The document includes a new recommendation for testing the aerosol containment of a given cell sorter with fluorescent beads before doing a potentially infectious sort.

Fixed Cell Sorting
Demand for unfixed cell sorting has increased and assays involving cytokines or apoptosis, live DNA or RNA staining, and various membrane studies cannot be done with fixed cells (Schmid et al., 2007). However, if it is feasible for the experimental goals, fixation would be the biosafety recommendation. ISAC cautions that fixative concentrations should be verified as effective against potential infectious agents. An example cited describes fixation of cells infected with Human Immunodeficiency Virus (HIV) with 1% paraformaldehyde. The titer of HIV in infected cells, or in infected blood, was greatly reduced by the 1% paraformaldehyde; however, cell-associated virus was not completely inactivated. It was possible to recover HIV from the fixed blood cells up to 18 hours post-fixation (Aloisio, 1990). In addition, even if the samples are completely inactivated by fixation, aerosol control may be a concern since the stains used may be toxic or carcinogenic (Schmid et al., 2007).

Guidance on Risk Assessment
Researchers are under tremendous pressure to answer pressing biomedical research questions and may challenge safety decisions prohibiting infectious cell sorts in shared cytometry facilities. The ISAC biosafety guidelines provide a framework for discussion of these conflicts. The risk assessment sections refer staff to the biosafety professional for review of the infectious, carcinogenic, or recombinant hazards of the samples, and review issues such as the cell sorting instruments to be used and the potential for discharge or aerosols and exposure to the operator or bystander. Engineering controls, personal protective equipment, medical surveillance, and the level of flow cytometry and laboratory experience required for risk minimization are also covered. In my experience, researchers balk when denied access to a cell sorter in a shared facility and point to the fact that a given BSL-2 infectious agent is not transmitted by aerosol in the general population. This can be countered only with the argument that cultures have a higher concentration of the infectious agent, and cell sorting requires the deliberate formation of droplets. Examples of agents, which can be transmitted by a different route in a laboratory setting, include airborne transmission of scrub typhus (Oh et al., 2001) and ingestion transmission of HIV (Ruprecht et al., 1999).

How are the aerosols formed?
A general explanation of the cell sorting process is available on many flow cytometry web sites such as www.unsolvedmysteries.oregonstate.edu/flow_cytometry_06.shtml. Aerosol generation occurs when a cell suspension is drawn up and pumped through a vibrating nozzle for the purpose of breaking the cell stream into individual droplets that fall between high voltage plates. The ISAC guidelines describe the creation of droplets and aerosols during cell sorting in detail. High-speed sorters operate under greater pressure and produce more small droplets as compared to older instruments that operate at slower speeds. However, the guidelines state clearly that "all sort-
ers also generate microdroplets, i.e., satellite droplet 3 to 7 μm (Schmid et al., 2007), and additional droplets of all sizes are produced when a nozzle becomes partially clogged or the stream is accidentally deflected onto a hard surface, such as the waste catcher.” Excellent photos of the aerosol/droplet stream created when the cell sort is not operating optimally on the web can be viewed at www3.interscience.wiley.com/cgi-bin/fulltext/104084100/HTMLSTART (Perfetto, 2003). For this reason, the ISAC guidelines include the following statement: When sorting any infectious or hazardous material, even if it is classified as BSL-2, it is critical to understand that droplet-based sorting procedures are considered BSL-3 practices. It is therefore recommended that viable, unfixed samples that are potentially infectious be sorted at a minimum on a sorter which has been tested for aerosol containment located in a BSL-2 facility (modified as described in Environmental Controls, described below) using practices and containment equipment for BSL-3 by the CDC. However, because of the increased hazard of a sudden quick release of large amounts of fluid or aerosols into the environment, it is highly recommended that high-speed sorting be performed in a BSL-3 laboratory facility under complete BSL-3 containment.

For BSL-2 sorts, ISAC recommends enclosing the cell sorter in a biosafety cabinet or enclosure. When that is not possible, the recommended environmental controls are:

- Cell sorters used for BSL-2 sorts should be located in a separate, lockable room where no other lab activity is performed.
- The room exhaust should discharge to the outside away from occupied areas or be HEPA filtered.
- Airflow in the room is balanced to no less than 10 changes of air per hour.

### Advising Researchers on Purchase or Upgrade of Aerosol Containment Capability

Some flow cytometers have been specifically designed to fit in a biosafety enclosure, and researchers ordering these devices may not be aware of this fact. In addition, containment improvements are available for many other flow cytometers (example in Figure 1). A careful review of instrument containment should be included in the risk assessment of cell sorting, and upgrades should be considered where appropriate. The "aerosol containment features" are auxiliary vacuum pumps to reduce aerosols in the chamber before the door is opened to address clogged nozzles or deflected streams. Some cell sorters are set up with a remote camera to allow the technician to observe the sorting streams away from the sorting area.

### Containment Enclosures

The BD FACSaria (Becton Dickinson Biosciences, San Jose, California) and JSAN (Bay Bioscience Co., Ltd. (Kobe, Japan) were designed to fit in a biocontainment biological safety enclosure (Baker Co., Sanford, Maine). A detailed explanation of this biosafety enclosure is presented in an ABSA Anthology (Ghidoni et al., 2006). The entire unit fits inside the Bioprotect II, some splash protection is also provided from the tubing that leads to the waste collection bottles. There are also high-speed sorters designed to be integrated into a biosafety cabinet: InFlux (Cytopeia, Seattle, Washington) and the Reflection (iCyt Visionary—Bioscience, Champaign, Illinois). Older units with water-cooled lasers (such as FACS Star, FACS Vantage, and FACSDiVa) may be too large to fit in a standard biosafety enclosure. However, a removable containment device (Cytek Development, Fremont, California) is commercially available. The Cytek unit draws air from the cell sorting and sample uptake areas and exhausts the air into the room through a HEPA filter. A custom enclosure would also solve an aerosol problem; Bigneat Containment Technology (Hampshire, United Kingdom) Flow Sciences, Inc. (Leland, North Carolina) and NuAire (Plymouth, Minnesota) have exhibited custom designs at ABSA conferences. One paper describes adapting the cell sorter to fit in a biosafety cabinet to allow sorting of peripheral blood cells in a BSL-2 laboratory (Lennartz et al., 2005). Becton Dickinson also recently purchased Dako Colorado, Inc. (Fort Collins, Colorado), which developed a Class I-type attachment for

### Figure 1

Example of biosafety options available for one model of flow cytometer, the Becton Dickinson FACS DiVa™, from the web site at www.bdbiosciences.com/immunocytometry_systems/products

<table>
<thead>
<tr>
<th>Closed Flow System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed flow channel for analysis of biohazardous samples</td>
</tr>
<tr>
<td>Autoclavable waste container with audible overfill alarm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BD™ Aerosol Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aids in aerosol management for high-speed sorters</td>
</tr>
<tr>
<td>Supplies distributed vacuum at the sort chamber when sort chamber door is closed</td>
</tr>
<tr>
<td>Displays visible filter integrity display</td>
</tr>
<tr>
<td>Is compatible with the BD FACSDiVa™ option</td>
</tr>
</tbody>
</table>

**Note:** Although these aids help with aerosol management, they do not replace good laboratory practices. All routine laboratory biological hazards protection should be followed in conjunction with the aerosol management system.

<table>
<thead>
<tr>
<th>Sample Splash Shields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provide additional droplet containment around the sample inlet port and nozzle assembly area</td>
</tr>
</tbody>
</table>
the MoFlo high-speed cell sorter. However, please note that while these “containment systems permit sorting of materials classified as BSL-2 using BSL-2 practices, the effectiveness of aerosol containment should be verified through rigorous testing before sorting any potentially infectious samples” (Schmid et al., 2007).

Auditing Safety Measures

This new ISAC Standard emphasizes the importance of audits to verify that containment measures are effective. ISAC advises:

- Testing the sort chamber with bottled smoke and sealing any leaks found
- Using splash shields for the sample uptake area and/or the sort chamber
- Accessing and inspecting fluidic tubing that is under pressure before infectious sorts
- Using sizing nozzles appropriate for the cells to be sorted. At a minimum, the aperture should be four times greater than the cell size; six times the size of the cells to be sorted is listed as the ideal (Schmid et al., 2007).

Some manufacturers recommend cleaning the nozzles by sonication—biosafety professionals will have opinions on where that is done! Details such as proper disinfection of the equipment according to manufacturer’s directions, sink discharge of disinfected waste, and the use of personal protective equipment and handwashing after removal of personal protective equipment should all be evaluated during an audit. If a risk assessment warrants the wearing of respiratory protection, staff should be enrolled in a respiratory protection program. The ISAC standard contains a great deal of substantive, updated information that will be of assistance to biosafety professionals whose responsibilities require them to review the sorting of unfixed biohazardous samples. Sharing the ISAC guidelines with staff performing this procedure is an important first step for safe cell sorting.

References


Molecular Biosafety

Margy S. Lambert

University of Wisconsin—Madison, Madison, Wisconsin

The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at mlambert@fpm.wisc.edu or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

A Reassessment of Adeno-Associated Virus Vector Risks That Takes New Information on Insertional Mutagenesis into Account

A recent Science article implicated insertional mutagenesis as a potential mechanism for induction of liver cancer in mice by adeno-associated virus (AAV) vectors (Donsante et al., 2007). The results of this study, as discussed by Kay 2007, have resurfaced concerns that AAV vectors may not be as safe as previously thought and may influence how AAV and recombinant AAV will be handled in research laboratories in the future.

AAV is a parvovirus that can be aerosol-transmitted and a dependovirus that normally requires another virus such as adenovirus or herpes simplex virus (HSV) to supply factors that support replication (Hamilton et al.,
2004). AAV is generally considered nonpathogenic; therefore, AAV has been assessed as a risk group 1 (RG1) virus with the use of biosafety level 1 (BSL-1) conditions considered adequate (NIH Guidelines for Research Involving Recombinant DNA Molecules: www4.od.nih.gov/oba/rac/guidelines/guidelines.html).

On the other hand, some characteristics of AAV may increase risks, including the following: detection in embryonic tissue (Burguete et al., 1999; Dutheil et al., 1997; Kiehl et al., 2002), an association with male infertility (Erles et al., 2001; Mehrle et al., 2004), the ability to replicate in some cases without a helper virus (Meyers et al., 2000), and the capability of integrating into the host genome (Miller et al., 2002). AAV is an unusual virus that can not only integrate into the host genome, but also is inserted preferentially at a specific site (on human chromosome 19). AAV integration translates to AAV having the potential for insertional mutagenesis, and for latency where AAV can be reactivated to a productive infection at a later time when a helper virus is present.

Based on risk information available before the 2007 Donsante article was published, the University of Wisconsin-Madison’s Institutional Biosafety Committee (IBC) instituted a policy in 2006 (modified in early 2007) deeming that some procedures involving AAV and AAV vectors should be conducted using biosafety level 2 (BSL-2) precautions and containment (www2.fpm.wisc.edu/biosafety/ibc/docs/OpportunisticPathogens_Jn07final.pdf).

Earlier research conducted by the authors of the 2007 Science article indicated the same type of results: liver cancer in mice associated with AAV vector treatment (Donsante et al., 2001). The main drawbacks of this study were the evaluation of only one knockout strain of mice and small numbers. This article raised sufficient concerns; however, that the Food and Drug Administration (FDA) temporarily halted two clinical trials using AAV vectors in 2001.

Some research studies indicate that there is no association between AAV vectors and hepatocellular carcinoma (HCC). One significant research paper, in particular, concluded that there was no evidence for AAV vector induction of liver cancer in a large-scale study in mice (Bell et al., 2005). This analysis was a compilation of many individual studies so a variety of different vectors, mouse strains, and experimental conditions were employed. One tumor was observed in 695 mice receiving AAV vectors while none were observed in the 226 control mice. The primary mouse strain used was C57BL/6, which is a strain classified as having a low frequency of spontaneous liver tumor formation. Drawbacks of this study included: using mice of a broad range of ages (it’s conceivable that more tumors would have appeared if the mice were allowed to age longer), evaluation of only part of each liver (an average of one third of each), and using a variety of AAV constructs including different transgenes and regulatory sequences. The authors recommend that future studies use mice matched for age, vector, dosage, strain, and other relevant parameters.

A symposium entitled “Safety Considerations in the Use of AAV vectors in Gene Transfer Clinical Trials,” jointly sponsored by the National Institutes of Health (NIH) and the FDA, was held in March 2001 (www4.od.nih.gov/oba/rac/Transcript3-7-011.pdf). The conflicting research results for an association between AAV vectors and liver cancer were discussed, and the NIH Recombinant Activities Committee (NIH RAC) concluded that the tumors observed in the 2001 murine liver cancer study were not likely caused by AAV vectors. It was suggested that the tumors were probably due to the knockout strain of mice used in the study being especially susceptible to cancer. As a result, the human gene therapy trials utilizing AAV vectors that had been put on hold were allowed to continue.

The 2007 Donsante study tested the association of liver cancer with AAV vectors using several strains of neonatal mice, including wild type mice, and found similar results as their previous research (56% or 33% of treated mice [depending on strains of mice and specific AAV constructs used] developed liver cancer as compared to 8%, or 4% for the untreated controls). In addition, the current study detected AAV vector integration events in the liver tumor tissue and mapped the integration site. Surprisingly, an evaluation of the tumors demonstrated that integration of AAV vectors was at a common site on mouse chromosome 12 rather than at random sites in the genome. For humans, altered gene regulation, including down regulation of a putative tumor suppressor gene at the syntenic (homologous) site on human chromosome 14 has been associated with several types of cancer (Astuti et al., 2005; Kawakami et al., 2006).

A common theory has been that if AAV genes (e.g., rep genes) that target integration to human chromosome 19 are removed, integration will occur at random sites in the genome (Kearns et al., 1996). Evidence from the 2007 murine liver cancer study contradicts that assumption. AAV vectors (at least in some tissues) appear to integrate preferentially at specific sites, potentially leading to altered regulation of nearby key genes and disease outcomes. This result could be due to either a site-specific recombination mechanism, or to random integration followed by selection for cells containing AAV vectors inserted at that site.

This research provides evidence that AAV vector insertional mutagenesis risk is higher than previously thought. Removing genes responsible for insertion of AAV at the preferred site on chromosome 19 does not eliminate the potential for insertional mutagenesis. In fact, insertion of AAV vectors at sites near high hazard genes may carry more risk than insertion at the chromosome 19 site preferred by wild type AAV containing functional rep genes.
Key points from the 2007 Science article are the following: 1) The results of the previous study were repeated and the criticism of the earlier study that the results would likely not apply to wildtype mice was refuted; 2) A mechanism for the association between AAV vectors and liver cancer (insertional mutagenesis) was postulated; and 3) the surprising result that AAV vector integration occurred at a specific site rather than at random sites in the host genome was observed.

What explains the conflicting results of different studies on the association of AAV vectors with insertional mutagenesis and cancer? This may be a case of, “The devil is in the details.” The specific experimental conditions of the study may define whether tumorigenesis occurs. Important factors that should be studied in more detail include: genetic susceptibility, AAV construct specifics including transgenes and regulatory sequences, characterization of AAV vector integration sites, involvement of DNA repair factors, tissue specificity, the presence of environmental triggers, such as DNA-damaging chemicals or radiation, and identification of disease genes near insertion sites with analysis of whether the expression of these host genes is altered.

The genetic background of different murine species could make a difference in whether AAV vectors can induce liver cancer through insertional mutagenesis. Other host variables such as age and gender could also influence whether liver cancer can be induced by AAV vectors. Thus, it may not be surprising that liver cancer was not seen in murine strains with a low rate of spontaneous tumorigenesis, while liver cancer was detected in other strains that have a higher susceptibility to carcinogenesis. A similar dichotomy is seen in humans with some individuals showing a higher tendency to get cancer due to their genetic background.

The 2007 Donsante study showed that the frequency of liver cancer varied depending on the AAV construct used: 56% of mice developed liver cancer when the b-actin promoter was present in the AAV construct, while 33% of mice developed liver cancer when this promoter was absent from the construct. In this case, the different frequencies of liver cancer occurred when the same transgene, but different regulatory sequences were present in the AAV constructs. The same research group that concluded there was no evidence for tumorigenesis of AAV vectors in a large-scale murine study in 2005, reported research indicating an association of tumor formation and liver cancer with AAV vector delivery to the liver, which was dependent on the transgene delivered. The authors conclude that the expression of some transgenes alone, or in combination with the AAV vector may be problematic (Bell et al., 2006).

Other studies support the Donsante 2007 article’s conclusion that AAV vector integration is not random. Actively transcribed genes are targeted in the liver (Russell, 2003). A large scale characterization of integration sites in fibroblasts (Miller et al., 2005) and murine liver (Nakai et al., 2005) shows preferential integration near transcription start sites and regulatory sequences; the presence of hotspots for integration, deletions and other rearrangements in host chromosomes at insertion sites; and a 3.5% integration frequency in cancer-related genes (in the liver). The Nakai study concludes that gain of function, in addition to loss of function mutations in disease-related genes, can be caused by AAV vector-mediated insertional mutagenesis.

The involvement of DNA repair in AAV integration needs further study. AAV appears to use some host DNA repair factors in the integration mechanism. Unlike retroviral vectors, the AAV integration mechanism exploits aspects of DNA repair pathways with integration at chromosomal double-stranded breaks (Russell, 2003; Miller et al., 2004). In vitro and in vivo studies indicate that inhibition of DNA repair enzyme DNA-PK results in an increased level of AAV integration (Song et al., 2003).

Different tissues show different patterns of AAV transduction and integration and of tumorigenesis. AAV vector integration in the liver has been shown to occur preferentially in actively transcribed regions of the genome (72%), to cause deletions and other rearrangements at chromosomal integration sites (100%), and 100% of the targeted genes analyzed were found to be genes expressed in the liver (Nakai et al., 2003).

DNA-damaging agents (chemicals, gamma radiation, and ultraviolet radiation) can increase the rate of AAV transduction (infection of cells) and the frequency of AAV integration (Alexander et al., 1994; Russell et al., 1995, Peng et al., 2000). Some DNA-damaging agents were able to induce transduction by 750-fold. The liver is, of course, the primary site for detoxification of chemicals so it is a tissue where the opportunity for chemical induction of AAV transduction and AAV integration would be greater.

If a disease association (e.g., cancer types other than liver) is detected in future studies involving AAV vectors, similar testing, as was done in the 2007 Science article, should be carried out. If possible, host chromosome insertion sites should be identified and expression levels of nearby genes analyzed. This sort of investigation could determine whether other disease outcomes are associated with AAV vectors, under what experimental conditions, and what disease genes are involved in the process.

A July 2007 death in a gene therapy trial using AAV vectors was investigated by NIH RAC with their findings detailed in their September 2007 and December 2007 meetings (webcast: videocast.nih.gov/default.asp). The main cause of death was apparently an overwhelming infection with Histoplasma capsulatum. The expert panel deemed it unlikely that gene therapy contributed to the death, but stated that this possibility cannot be ruled out definitively. A potential role for insertional mutagenesis was not discussed.

What does the above information mean for the bio-
safety community? In conducting risk assessments, investigators should be aware of the recent information on insertional mutagenesis. Factors considered in risk assessments of in vitro AAV vector projects should include: aerosol route of transmission, aerosol-generating activities, quantity and concentration of the virus, and transgene expressed. Additional factors evaluated for in vivo experiments should include: dosage, route of administration, organ targeted, and the ability of AAV vectors to migrate from the target site. Some tissues could present higher risks (e.g., the lungs, because of aerosol transmission, and the liver, because of the recent data indicating an association between AAV vector integration and liver cancer).

The 2007 Donsante study provides a compelling argument for insertional mutagenesis being a potential risk in AAV vector research. Based on this new information, IBCs may want to consider setting policies designating the use of BSL-2, rather than BSL-1 precautions and containment, for some research projects involving AAV vectors.

References


Capsule

Ed Krisiunas

WNWN International, Burlington, Connecticut

What’s new? What’s hot? What’s timely? If you don’t have time to search the Internet for the latest developments that might impact your work environment, you just might find some of this information in the “Capsule” column. Please e-mail any comments or suggestions to ekrisiunas@aol.com or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Effectiveness of Personal Protective Measures to Prevent Lyme Disease


The introduction states: “After the manufacture of Lyme vaccine was discontinued in 2002, strategies to prevent Lyme disease (LD) have focused on personal protective measures. Effectiveness of these measures has not been conclusively demonstrated.” The purpose of this study was to assess the effectiveness of personal preventive measures. The authors of the study concluded that use of protective clothing and tick repellents (on skin or clothing) is effective in preventing LD. Visit the following link for more information:

www.cdc.gov/eid/content/14/2/pdfs/07-0725.pdf

**Laboratory-acquired Brucellosis—Indiana and Minnesota, 2006**

In November 2006, two cases of brucellosis in microbiologists at two clinical laboratories were reported to state health departments in Indiana and Minnesota. The Minnesota Department of Health (MDH) contacted CDC regarding this suspected multistate cluster of laboratory-acquired brucellosis. MDH and the Indiana State Department of Health (ISDH) asked CDC to conduct further testing on *Brucella* isolates suspected of causing the infections and to provide recommendations for appropriate response by the laboratories. This report summarizes the investigation conducted jointly by MDH, ISDH, and CDC, provides guidance on safe laboratory handling of *Brucella* spp., and makes recommendations for responding to *Brucella* laboratory exposures. The results of that investigation determined that 146 workers at the two laboratories had been exposed to *Brucella* and that, although two *Brucella* isolates had been handled by both laboratories, infections in the two microbiologists were caused by two unrelated isolates. The events in Indiana and Minnesota emphasize the importance of adhering to recommended biosafety practices, timely sharing of information regarding laboratory exposures, and rapid implementation of response protocols. Visit the following link for more information:

www.cdc.gov/mmwr/preview/mmwrhtml/mm5702a3.htm

**MMWR: Recommended Immunization Schedules for Persons Aged 0-18 Years—United States, 2008**

The recommended immunization schedules for persons aged 0-18 years and the catch-up immunization schedule for 2008 have been approved by the Advisory Committee on Immunization Practices, the American Academy of Pediatrics, and the American Academy of Family Physicians. Visit the following link for more information:

www.cdc.gov/mmwr/preview/mmwrhtml/mm5701a8.htm

**Homeland Security Centers of Excellence**

The Homeland Security Centers of Excellence (HS-Centers) bring together leading experts and researchers to conduct multidisciplinary research and education for homeland security solutions. The centers are authorized by Congress and chosen by the Department’s Science & Technology Directorate through a competitive selection process. Each center is led by a university in collaboration with partners from other institutions, agencies, laboratories, think tanks, and the private sector. Visit the following link for more information:

www.dhs.gov/xres/programs/editorial_0498.shtm
Ask the Experts

John H. Keene

Biohaztec Associates, Midlothian, Virginia

Do you have a biosafety question and you’re not sure who to ask? Send your questions to the “Ask the Experts” column and I’ll get them answered for you. Drawing from my own experience or that of other experts in the field, we’ll try to compile a thorough and comprehensive answer to your question. Please e-mail your questions to jkeene@biohaztec.com or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Biosafety Cabinets, Containment Facility Maintenance, and More

Question

Even if a biosafety cabinet continuously passes certification tests against NSF49, should the filter be changed after a certain period of time? Is there an industry standard on this? Is gasket shrinkage a concern after 10 or even 20 years?

Answer

If the biosafety cabinet passes the NSF testing each year, there is no problem and the filter does not have to be replaced. Any damage to the gaskets would be recognized during the testing and, if found, would constitute a reason to replace the filter. Filters actually work better with time. In labs that are clean, I’ve personally seen biosafety cabinets in continuous operation for over 10 years with no problems. Yearly testing and clean labs make for safe cabinets.

Question

I have a facilities maintenance group that is panicking over decontamination of some secondary containment systems in our BSL-3 containment facility. The manufacturer has not been helpful in assisting in the decontamination process and does not appear to be concerned that some areas of the equipment may need to be decontaminated and serviced. Facilities maintenance wanted to know who would decon these systems and the vendor was not too accommodating. Should we develop a decon plan of our own, or should we try to get the vendor to provide the service?

Answer

This is a problem that has two aspects and that seems to come up only after the facility has been in operation for a period of time. The two areas of concern are: 1) perception of maintenance personnel that all areas of biocontainment labs are contaminated and decontamination is difficult and risky; and 2) some vendors are interested in selling their product and moving on without reviewing the potential hazards associated with the need to repair the equipment.

It is important in the start-up of any biocontainment laboratory to involve and train the personnel who will be responsible for the preventive maintenance regarding the actual probabilities of contamination of systems and how to handle their decontamination. This means that the biosafety professional must be proactive in ensuring that a program be in place to cover any contingencies with regard to the repair of the critical systems in the containment laboratory. This program must cover not only information regarding the potential for contamination, but also the mechanisms/protocols for decontamination of the systems and equipment. In addition, it should be evident to the maintenance personnel that the containment facility management is concerned about protecting them from potential exposure. Such a program must include training scientific personnel regarding the operation of the facility and the need for strict adherence to the safety protocols while working in the facility.

With regard to the second point, vendors must be held accountable for ensuring that the equipment they manufacture and sell can be appropriately decontaminated and serviced. A review of the operation of the systems and equipment by the biosafety professionals early in the process of design and acceptance by management that a risk assessment must be performed will assist in minimizing the problems that invariably arise after the facility is in operation. Vendors must be able to answer the tough questions posed by the reviewers and provide reasonable answers or solutions to questions posed. When operating a biocontainment facility, it is unacceptable to purchase from a vendor that cannot provide answers and solutions to questions regarding the preventive maintenance of its product.

Prior to initiating work in a new biocontainment facility, it is imperative that the facility management, in conjunction with the biosafety professional, develops the appropriate contingency plans for decontamination and repair of critical equipment. Waiting until something catastrophic happens to develop such plans will result in loss of research time and increased costs, not to mention loss of credibility with the maintenance personnel required to keep the facility operational.
**Question**

When removing PPE as an individual is leaving a BSL-3 laboratory room, the process of taking off a Tyvek suit/foot covers properly is a challenge. Can a scientist’s street shoes ever touch the floor of a BSL-3 laboratory or can this contact occur only in a “gray zone” adjacent to the exit door? Can bare hands ever touch the handle of the exit door? Can a scientist sit down on a chair in a BSL-3 suite while removing PPE and have his/her street clothes in contact with the chair (which is the chair at a Class II BSC)?

**Answer**

You bring up some good points that need to be addressed because there are misconceptions about biocontainment labs. First, these laboratories are not contaminated unless a spill has occurred outside the biosafety cabinet, so the use of excessive PPE is not generally warranted in the lab. Certainly, things like foot covers are not necessary and even the use of Powered Air Purifying Respirators (PAPRs) may be excessive since the work is supposed to be performed within the primary containment device, the BSC.

In my experience, about 10% of the people using biosafety cabinets actually know how they work and the limitations of working in them. In over 20 years of teaching laboratory safety, I have given classes a quiz asking how many of the students have BSCs in their labs and how many of them know exactly how the BSC works. During this time about 10% of the students actually know the answer. It is probably good that folks are wearing PPE. However, it is more important that we teach them how to use the primary safety equipment and reduce the reliance on PPE. That is the OSHA position on this issue as well.

So to answer your questions:

1. Tyvek suits and shoe covers are probably overkill in most BSL-3 labs—they are not necessary.
2. BSL-3 labs are not contaminated rooms; anyone can walk through them without fear of contaminating his or her shoes.
3. Yes, bare hands can touch the handle of the door to the room because it is not contaminated unless someone working in the lab has touched the door handle with contaminated gloves—another training and supervisory issue.
4. Yes, a scientist or anyone else who has permission to be in the lab can sit down and have contact with any chair in the lab. The chairs should not be expected to be contaminated.

In the absence of a catastrophic spill or accident outside of primary containment, BSL-3 facilities are clean areas. One thing we forget when discussing BSL-3 labs is that these labs generally are working with agents that are present in the natural environment, not exotic “deadly” agents. Our emphasis should be on a realistic risk assessment and ensuring that the procedures are correct, reasonable, and followed. The engineering safety devices are only as good as the folks who are using them.

---

**Free Downloads from the Journal Human Gene Therapy**

If your Institutional Biosafety Committee reviews human gene transfer trials, members may want to review the commentaries, editorials, and reviews that are available as free downloads from the Journal Human Gene Therapy at www.liebertonline.com/toc/hum/19/1. Some downloads include:

**Commentaries**

“Case of Leukaemia Associated with X-Linked Severe Combined Immunodeficiency Gene Therapy Trial in London”
“If It’s Broken, Shouldn’t It Be Fixed? Informed Consent and Initial Clinical Trials of Gene Therapy”
“Informed Consent in Human Gene Transfer Clinical Trials”
“Protections for Participants in Gene Therapy Trials: A Patient’s Perspective”
“Human Gene Therapy, Consent, and the Realities of Clinical Research: Is It Time for a Research Subject Advocate?”

**Editorial**

“Adverse Events in Gene Transfer Trials and an Agenda for the New Year”

**Review**

“Advances in MicroRNAs: Implications for Gene Therapists” by Rebecca T. Marquez and Anton P. McCaffrey
Since the September 11 attacks in 2001, Battelle, with the nation’s largest privately owned BSL-3 laboratory, has seen continuous and substantial growth due to an increased interest in Select Agent research. Battelle conducts the bulk of this type of research at its 183,000+ sf Biomedical Research Center (BRC) in West Jefferson, Ohio, 17 miles from the organization’s headquarters in Columbus.

Research at the BRC focuses on developing medical countermeasures against pathogens, decontamination of toxic materials, and threat assessment. The facility now houses more than 180 scientific and support personnel, up from only 61 staff members five years ago. The BRC is just one of several BSL-3 facilities in the country capable of studying aerosolized biological agents in living organisms.

The newest addition to Battelle’s BRC is a 53,000-sf, three-story facility connected to three other existing research buildings by airlocks and hallways. The latest facility includes six new ABSL-3 animal holding rooms, bringing the total for the entire complex to 15, and 10 new BSL-3 labs, increasing the total to 22. Office space in the new facility is wrapped along the outside corridor, providing cubicle or office space for 90-plus staff members.

The Schedule

“We were in a good position to accelerate this project schedule since the new facility was built to complement and expand on existing facilities,” says John Henneman, coordinator for Battelle’s BRC Biofacilities, who served as onsite project director for construction of the new facility. “We knew our equipment requirements upfront and had a good idea of what we wanted in terms of structural components.”

The project took approximately 20 months from initial design to occupancy. In March of 2005, Battelle hired the Gilbane Building Company of Columbus, Ohio, to serve as construction manager. Construction started in January of 2006, with occupancy and move-in beginning in August of that same year.

“Most likely we shortened the normal process by about three or four months by establishing Gilbane as CM-at-risk as the first step, before even selecting a project architect,” says Henneman. “It was a departure from the traditional design process, but for this project it made sense since it allowed Gilbane to start buying major equipment right away that we knew we needed such as decontamination autoclaves and cagewashing systems.”

“Even after the full team was in place we tried to incorporate strategies throughout the construction process to keep the project schedule ahead of pace,” says Jeff Schramm, vice president of Gilbane and project executive for the project.

“For example, instead of having traditional submittals merely forwarded to our office, we threw what we called ‘submittal parties’ at the construction site where vendors and subcontractors could submit their bids in person,” says Schramm. “This let us go through submittals immediately and respond to subcontractors right away.”

Facility Highlights

“In contrast to our existing labs, individual holding areas in the new facility are located directly across the hall from six of the BSL-3 labs,” says Henneman. “It is also designed so that each end of the facility has its own changing rooms, autoclave, and cage washing areas to address redundancy issues.”

While Battelle prefers to keep most of its actual laboratory configurations and practices proprietary, Henneman was able to describe several unique safety features within the labs.

“The Class-III biosafety cabinets in use within the new BSL-3 labs were developed specifically for Battelle in 2004,” says Henneman. “They were the first ever Class-III biosafety cabinet to be produced with mobile transfer carts.”

“Over the years we have made modifications to the original design including the addition of rapid transfer ports that allow us to conduct aerosol research within the cabinets in addition to low-containment studies.”

Henneman points to the facility’s underground access tunnel as another distinctive feature of the new facility.

“We minimized the space underneath the building by only allowing access to the piping and the critical areas that lead to our effluent decontamination system,” says Henneman. “The utility tunnels are positioned directly below corridors that service holding rooms and labs providing easy access to the pipes for maintenance.”

Large viewing windows were added to six of the new...
labs so that other researchers or visitors can observe lab activities from the hallway. Of these labs, three also have a wireless intercom system that enables tours to also hear what is happening in the lab and enhances communications between technicians and project leaders.

Animal holding areas in the new facility all use continuous-sealed MMA flooring and have sloped trench drains along the floor level of the interior walls. The drains are protected by a rail system to ensure that animal racks cannot roll into the drains. In addition, the animal holding rooms have adjacent 8' x 12' anterooms to further isolate animals from the facility’s clean corridor.

CDC Approval

The new labs and containment areas are currently operating as a BSL-2 facility since the results of Battelle’s CDC Select Agent inspection that took place in October of 2006 are still pending.

“We fully expect to be operating as an enhanced BSL-3 by the middle of 2007 once the paperwork and CDC registration is complete,” says Henneman. “We designed the facility to meet all requirements of enhanced BSL-3 laboratories as defined by CDC’s guidelines in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) publication.”

These enhanced requirements include security access control and monitoring, gowned areas for labs and shower out capabilities, autoclaves for solid waste, liquid effluent decontamination systems, and gas decontamination capabilities for entire labs.

The new facility has pass-through autoclaves that are eight feet long, 50 percent larger than autoclaves in use in other areas of Battelle’s BRC. In addition, the new facility has hydrogen peroxide vapor (HPV) ports in the ceiling so that the entire lab can be decontaminated with peroxide gas. Over the last 11 years, Battelle has used validated HPV technology to safely remove critical equipment from the containment laboratory.

“In addition to meeting the CDC’s Select Agent requirements, every decision we made was related to the science of the building,” says Henneman. “As we continue to expand our work with Select Agents and vaccine research, we needed this new facility to be able to handle any animal research requirements related to rodents, non-human primates, and poultry.”

Lessons Learned

“Integrated involvement between the owner and among all team members from the very beginning of the project is what allowed us to accomplish what we did in such a short period of time,” says Schramm. “It is also what helped us ensure that no major shutdowns took place in the adjacent BSL-3 animal facility that stayed in operation during the entire construction process.”

He adds that even the independent commissioning agent was brought on board at the very beginning of this project. It is a strategy he recommends for all major projects, rather than reserving commissioning as an end-of-construction task.

“Having the commissioning agent attend our monthly planning and update meetings ultimately saved an immense amount of time,” says Schramm. “Originally we planned that commissioning would take a couple of months at the end of the job. In reality it took only two and a half weeks because we had worked so collaboratively throughout construction.”

Schramm also recommends working closely at the start of each project with mechanical and electrical sub-contractors to coordinate utility issues including critical elements such as where ductwork will be positioned, how conduit will be mounted, how light fixtures will be mounted, how supply air will be diffused, and how all of those things will tie together.

“All conduit in Battelle’s new facility, including lighting, is surface mounted which saved us time during construction because we did not need to coordinate with mechanical engineers about placing and sealing the conduit within the walls,” says Schramm. “Surface mounting is equally effective and more efficient when timing is an issue.”

Henneman feels that a key element to this project’s success was that the final building design is flexible enough to respond to evolving scientific programs and equipment upgrades.

“We wanted a facility that can easily adjust to future research needs,” says Henneman. “Our current researchers have exactly what they need now, but we also added things like extra utilities, drainage, and plumbing so that the rooms are flexible enough to accommodate different types of equipment, if necessary. We can easily switch gears for different research needs.”

Biographies

John Henneman serves as coordinator of special facilities for Battelle’s BRC. He has more than 25 years of experience in scientific research of which the last 12 have been managing biofacility operations of the BRC. He began his career as a lab technician and research associate for the National Cancer Institute. In his current position, Henneman controls facility access, coordinates numerous maintenance contracts, supervises water testing, manages infectious waste facility operations, and chairs the BRC Environment, Safety, Health and Security Committee. Henneman has a Bachelor’s degree in biology from Mansfield University, Mansfield, Pennsylvania, and a Master’s degree in environmental biology from Hood College, Frederick, Maryland.

Jeff Schramm is a vice president and principal of Life Sciences COE (Center of Excellence) for the Gilbane
Building Company. Schramm has worked in the construction industry for 23 years. He is responsible for Gilbane’s Life Sciences Center of Excellence, which serves clients that are constructing biocontainment, high-tech laboratory, pharmaceutical and biotechnology facilities in the private, public, and university sectors.

This article is based on a presentation given by Henneman and Schramm, along with David Duthu, at the Tradeline Animal Research Facilities conference in November 2006.

For more information, please contact John Henneman, Special Facilities Coordinator, Battelle, 505 King Avenue, JM-7, Columbus, Ohio 43201-2693, 614-442-4575, henneman@battelle.org, or Jeff Schramm, Vice President, Gilbane Building Company, 7901 Sandy Spring Road, Suite 500, Laurel, Maryland 20707, 301-317-6118, jschramm@gilaneco.com, or David Duthu, PE, Board Principal, ccrd principals, 808 Travis Street, Suite 200, Houston, Texas 77002-5706, 713-237-8900, davidd@ccrd.com.

**Figure 1**

Battelle Memorial Institute, which conducts $3.7 billion in annual research and development, recently opened this new BSL-3 lab and animal holding facility at its Medical Research and Evaluation Facility (MREF) in West Jefferson, Ohio, 15 miles from the organization’s headquarters in Columbus. (Photo courtesy of John R. Henneman, Battelle.)

**Figure 2**

These mobile Class-3 biosafety cabinets include glove ports and rapid transfer ports so that Battelle researchers can conduct both low-containment studies as well as high-containment aerosol research studies. (Photo courtesy of John R. Henneman, Battelle.)

**Pandemic Flu Epidemic Web Site**

Managed by the United States Department of Health and Human Services, the following web site provides information to assist individuals and communities in preparing for a pandemic flu epidemic:

www.pandemicflu.govtaketheleadindex.html
2007 ABSA Service Award Recipients

Award Presentations from the ABSA Conference, October 2007

Arnold G. Wedum
Distinguished Achievement Award
Joseph Songer
Ames, Iowa

The Arnold G. Wedum Distinguished Achievement Award is given to a current ABSA member for outstanding contributions to biological safety accomplished through teaching, research, service, or leadership.

Dr. Songer, a Charter Member of ABSA, was the first recipient of the Everett Hanel, Jr. Presidential Award, and served as president of the Association in 1988. Dr. Songer worked at the National Animal Disease Laboratory, Agricultural Research Service, U.S. Department of Agriculture (USDA), in Ames, Iowa where he conducted research on the effects of relative humidity and temperature on the survival of biological aerosols. His work was critical in the design, construction, and operation of some of the first high containment facilities engaged in activities with infectious diseases, especially those involving livestock. He created the Biosafety Bibliography containing important and original references of fundamental work prior to Internet availability of published investigations. Dr. Songer is a true pioneer whose many contributions provided the foundation for the development of the field of biosafety.

Everett Hanel, Jr.
Presidential Award
Daniel F. Liberman, PhD
Norvartis Institutes for BioMedical Research
Cambridge, Massachusetts

The Everett Hanel, Jr. Presidential Award is given to a current ABSA member for outstanding contributions to ABSA by promoting the field of biological safety and fostering, by example, the high professional standards of the Association’s membership.

Dr. Liberman is presented this award for his tireless work as a Charter Member of ABSA, and his consistent and continuing support of the organization. He has volunteered as a member of the Local Arrangements Committee (1995, 2006), as the chair of the Scientific Program Committee (2006-2007), and served on the committee in 1994. He has taught workshops for ABSA and other professional organizations, has been the lead editor of the Biohazard Management Handbook, and served on numerous governmental committees to shape biosafety guidelines and regulations. He receives the Everett Hanel, Jr. Presidential Award for his outstanding contributions to biological safety through teaching, research, service, and leadership.

Richard C. Knudsen
Memorial Publication Award
Mark A. Czarneski
ClorDiSys, Inc.
Lebanon, New Jersey

The award shall be given, when merited, to the author(s) of an article that reports a significant contribution in scientific investigation and/or health and safety in those areas of interest to Richard Knudsen during his career. The award recipient need not be a member of the American Biological Safety Association.

The Richard C. Knudsen Memorial Publication Award is presented to Mark A. Czarneski for his article entitled, “Selecting the Right Chemical Agent for Decontamination of Rooms and Chambers,” which was published in Applied Biosafety (Volume 12, Number 2, 2007, pp. 85-92). This article was selected as an excellent example of a very well-written review of the use of several commonly employed chemical agents, the benefits and deficits of their application, the methods for their use, and an outline of the factors to be considered in their selection. The contents of this article are of practical assistance to the diverse ABSA membership.

EPA Free Online Course

The EPA provides a free, basic online course entitled “Introduction to Mold and Mold Remediation for Environmental and Public Health Professionals.” For more information, visit: www.epa.gov/mold/moldcourse/chapter1/home.html
2007 ABSA Conference Sponsors

We would like to thank the following sponsors of the 2007 ABSA Conference in Nashville.

**Gold Sponsors**

**Germfree Labs, Inc.**, Ormond Beach, FL  
www.germfree.com

Germfree Labs, Inc. manufactures primary and secondary containment equipment found in BSL laboratories with our focus being on Class III BSC and transfer carts. Germfree’s product line includes turnkey mobile and modular laboratories in which we retain manufacturing control of all critical primary and secondary containment equipment allowing us to provide a completely integrated laboratory of the highest quality.

**Novartis**, East Hanover, NJ  
www.novartis.com

Novartis wants to discover, develop, and successfully market innovative products to prevent and cure diseases, to ease suffering, and to enhance the quality of life. Our name, derived from the Latin novae artes, means “new skills” and reflects our commitment to focus research and development to bring new healthcare products to the patients and physicians that we serve. Created in 1996 through the merger of Ciba-Geigy and Sandoz, Novartis is currently organized into four divisions. We are always strengthening our medicine-based portfolio, and investing in strategic growth platforms. And Novartis is the only company with leadership positions in both patented and generic pharmaceuticals.

**Silver Sponsors**

**Diversified Laboratory Sciences, Inc. (DLS)**, Newnan, GA  
www.dlsconsultants.com

DLS/AED is a provider of laboratory consultative services worldwide and is comprised of dedicated professionals with over 80 years of combined laboratory bench work, biosafety management, design, and construction administration experience for BSL-2, BSL-3, and BSL-4 laboratories. Our services include laboratory planning/design, biosafety training/education, facility assessments, and SAP application assistance.

**Safety Plus, LLC**, Lookout Mountain, TN  
www.safetyplusllc.com

Safety Plus, LLC is a certified woman-owned business, specializing in bio-safety cabinet, laminar flow and cleanroom certifications, repairs, and reporting, while providing standardization. Our team of professionals provides services that will save time and money and impart a safer, more efficient work environment. Offices located in Georgia, Florida, and Tennessee.

**Siemen’s Building Technologies**, Buffalo Grove, IL  
www.sbt.siemens.com

As a leading provider of building controls, fire safety and security system solutions, Siemens Building Technologies, Inc., makes buildings comfortable, safe, productive and less costly to operate. The company focuses on improving the performance of its customers’ buildings, so that its customers can focus on improving their business performance. With U.S. headquarters in Buffalo Grove, Illinois, Siemens Building Technologies employs 7,500 people and provides a full range of services and solutions from more than 100 locations coast-to-coast. Worldwide, the company has 29,000 employees and operates in more than 42 countries.

**Bronze Sponsors**

**B&V Testing, Inc.**, Waltham, MA, and Bel Air, MD  
www.BandVTesting.com

Headquartered in the Life Science hub of Boston with service offices in the Mid-Atlantic, B&V Testing provides comprehensive testing, certification, decontamination, and repair services of cleanrooms and controlled environments, biological safety cabinets, chemical fume hoods, clean benches, isolators, HEPA filter banks, and animal care equipment for the pharmaceutical, biotechnology, medical device, healthcare and biomedical academic research communities.

**The Baker Company**, Sanford, ME  
www.bakerco.com

The Baker Company designs and manufactures the highest quality Class II and Class III Biological Safety Cabinets, Clean Benches, and fume hoods. Baker cabinets are designed for maximum ergonomic comfort, safety, and low life cycle costs. You can trust The Baker Company for safe solutions to all of your applications.

**Bio-Response Solutions, Inc.**, Danville, IN  
www.biresponsesolutions.com

Bio-Response Effluent Decontamination Systems (EDS) are designed to meet bio-containment facility design requirements. But, the cutting edge design goes beyond meeting specifications to delivering Biological Safety, pharmaceutical grade quality, and long life operation, while providing unparalleled safety to those working in the facility and to those who must service the equipment during its entire, long life.
Certek, Inc., Raleigh, NC  
www.certekinc.com
Certek is dedicated to creating products for the safe handling of hazardous substances. The products that have been developed are Modular Containment Laboratories, BSL2, BSL3, and BSL3+, plus the new animal laboratories, level 1 through 3, formaldehyde generator/neutralizers; SAFEMOD containment air filtration system and its companion, SAFESCAN, a system for in-place scan testing of HEPA filters.

Cornerstone Commissioning, Inc., Boxford, MA  
www.cxhvac.com
Cornerstone Commissioning, Inc. is a commissioning service provider for biocontainment and biomedical facilities. Our expertise is in leading and coordinating project teams through integrated system testing and successful turnover of projects to owners. Clients include USDA (BSL3Ag), NIH-Bethesda, Cornell, Harvard, Lawrence Livermore Laboratory and NIAID Regional Biocontainment Labs.

Midwest Research Institute, Kansas City, MO  
www.mriresearch.org
MRI performs contract research and laboratory consulting services in the areas of national security and defense, life sciences, food and agriculture, transportation safety, energy, and environment. With headquarters in Kansas City, MRI also has facilities in Palm Bay, Florida, Frederick, Maryland, and Rockville, Maryland. MRI has managed the National Renewable Energy Laboratory (NREL) in Golden, Colorado, for the U.S. Department of Energy since 1977.

National Biosafety & Biocontainment Training Program (NBBTP), Atlanta, GA  
www.nbbtp.org
The NBBTP offers stipend, full-time, two-year post-baccalaureate and post-doctoral biosafety and biocontainment training fellowships at the NIH Bethesda, Maryland campus as well as Certificate and Professional Development Courses for operations and maintenance and bio-safety and biocontainment personnel working in high containment facilities.

Nuaire, Inc., Plymouth, MN  
www.nuaire.com
Nuaire has been universally recognized as a leader for more than 35 years in providing laboratory professionals with reliable products such as biological safety cabinets, CO2 incubators, Laminar Airflow equipment, animal facility products, and ultra-low temperature freezers for the most demanding environments.

Recovery, Inc., Dupe, IL  
www.pri-bio.com
Progressive Recovery, Inc. is the global leader in engineering and manufacturing Biowaste/Effluent Decontamination Systems and now a provider of Caustic Digestion Units. PRI’s systems represent the final boundary and sterilization treatment of wastes before entering the environment. Uninterrupted laboratory performance, decontamination of pathogens and safety are recognized with PRI’s name and history.

Tecniplast USA, Exton, PA  
www.tecniplastusa.com
Tecniplast specializes in the design, manufacture, and installation of a broad range of products for the Life Science Community. Our product line includes a comprehensive selection of Housing, Washing, Laminar Flow, and Aquatic Systems. The SealSafe Plus and Isocage Systems offer the highest level Bio-Containment and Bio-Exclusion Security.

Thermo Scientific, Asheville, NC  
www.thermo.com/bsc
Thermo Scientific represents Thermo Fisher Scientific’s broad range of high-end analytical instruments, chemistry and consumable supplies, laboratory equipment, software and services. See the new Thermo Scientific 1300 Series Class II, Type A2 Biological Safety Cabinet. The 1300 Series delivers a smart design to ensure maximum safety, ergonomics, and energy efficiency.

Online Journal Submission Site

Applied Biosafety: Journal of the American Biological Safety Association is a peer-reviewed, scientific journal committed to promoting global biosafety awareness and best practices to prevent occupational exposures and adverse environmental impacts related to biohazardous releases. The goal of Applied Biosafety is to provide a forum to exchange and promote sound biosafety and biosecurity initiatives through the publication of new research in biosafety, as well as information on best biosafety practices, policy issues and position papers, editorials, commentaries, and reviews.

The new online submission site for Applied Biosafety is user-friendly and available at www.xcd.com/absa/article.cfm. If you have any questions, please contact the Production Editor, Karen Savage, at the ABSA Office at 1-866-425-1385 (toll free) or 847-949-1517 or via e-mail at karen@absa.org.
New ABSA Members for 2008

Namazbek Abdikykerimov
KRILVP, Mag
Kyrgyzstan

Tasneem Adam Ali
Univ. of Karachi
Pakistan

Rahmat Setya Adji
Balitvet
Indonesia

Syed Tanvir Ahsan
Aga Khan Univ.
Pakistan

Abdulwahab Al Kuhlani
High Inst. of Health Sciences
Yemen

Ahmed L. Al-Amari
Yemen National Ctr. of Public Health Lab.
Yemen

Asho Ali
Aga Khan Univ.
Pakistan

Samardin Aliev
Republican Ctr. of State Sanitary and Epidemiological Surveillance
Takikistan

Abdulrahman Alkateeb
Central Veterinary Lab.
Yemen

Khaled Al-Shaiban
Yemen National Ctr. of Public Health Lab.
Yemen

Karen Angel
Vanderbilt Univ.
Nashville, TN

Masood Anwar
National Inst. of Health
Pakistan

Elman Aslanov
RVL
Azerbaijan

Mamedyar Azaev
VECTOR
Russia

Ilham Azimov
State Veterinary Medicine Scientific Control Inst.
Azerbaijan

Eric Baillie
Univ. Health Network
Toronto, Ontario, Canada

Lela Bakanidze
National Ctr. for Disease Control & Medical Statistics of Georgia
Georgia

Sergey Balakhanov
Irkutsk API
Russia

Wanny Basuki
Eijkman Inst.
Indonesia

Regina P. Berba
Univ. of the Philippines
Manila, Philippines

Halyna Biletska
Lyiv RIEH
Ukraine

Alisha Blue
US CRDF
Arlington, VA

Vladmir Borisov
Vladmir, Russia

Lisa Brindel
Georgia Inst. of Technology
Atlanta, GA

Bakyt Burabayev
Research Inst. of Biosafety Problems
Kazakhstan

Svetlana A. Chubnidze
National Ctr. of Disease Control & Public Health
Georgia

Patrick Conley
Univ. of Texas Southwestern Medical Ctr.
Euless, TX

Magdalena Cruz
Bureau of Animal Industry
Philippines

Howard Cutler
Clean Air Flow, Inc.
Lincolnshire, IL

Iryna Demchysyna
SES
Kyiv, Ukraine

Ilya Drozdov
VECTOR
Russia

Shiv Chandra Dubey
High Security Animal Disease Lab.
Bhopal, India

Ivan Dyatlov
Obolensk
Russia

Mohamed Diaa El Din
National Research Ctr.
Egypt
<table>
<thead>
<tr>
<th>Name</th>
<th>Country/Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bozorboi Elmuradov</td>
<td>Uzbekistan</td>
</tr>
<tr>
<td>Olha Halyabar</td>
<td>Lyiv RIEH</td>
</tr>
<tr>
<td>Asankadyr Junoshov</td>
<td>Biotech Inst., NAS</td>
</tr>
<tr>
<td>Elham Fathy Azab Ahmed Elzoghby</td>
<td>Egypt</td>
</tr>
<tr>
<td>Ministry of Agriculture</td>
<td>US CRDF</td>
</tr>
<tr>
<td>Lisa Hilton</td>
<td>Arlington, VA</td>
</tr>
<tr>
<td>Jimmy Kalianda</td>
<td>DIC</td>
</tr>
<tr>
<td>Dan Endicott</td>
<td>Marcus Hodges</td>
</tr>
<tr>
<td>Univ. of North Florida</td>
<td>NIH/NBBTP</td>
</tr>
<tr>
<td>Soenjai Kamolsiripichaiporn</td>
<td>National Inst. for Animal Health</td>
</tr>
<tr>
<td>Keisha English</td>
<td>Suzanne Howard</td>
</tr>
<tr>
<td>Johns Hopkins Univ.</td>
<td>Wellesley College, EH&amp;S</td>
</tr>
<tr>
<td>Abdyla Kanybaev</td>
<td>Osh Regional Branch of RCQEDI</td>
</tr>
<tr>
<td>Hala Mohamed Esmat</td>
<td>Kineka Hull</td>
</tr>
<tr>
<td>Central Health Lab.</td>
<td>Wake Forest Univ.</td>
</tr>
<tr>
<td>Karamat A. Karamat</td>
<td>Planning Commission</td>
</tr>
<tr>
<td>Amber Farooqui</td>
<td>Xu Houjo</td>
</tr>
<tr>
<td>Univ. of Karachi</td>
<td>Shanghai Dowell Purity Engineering Equipment Company, Ltd.</td>
</tr>
<tr>
<td>Shahana Kazmi</td>
<td>SES, MOH</td>
</tr>
<tr>
<td>Nikolay Fedorov</td>
<td>Ismayil Huseynov</td>
</tr>
<tr>
<td>FGU Central Blood Transfusion Station</td>
<td>New City, China</td>
</tr>
<tr>
<td>Kalia Kasymbekova</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Valentina Fedorova</td>
<td>Aamer Ikram</td>
</tr>
<tr>
<td>Saratov State Univ.</td>
<td>AFIP</td>
</tr>
<tr>
<td>Adnan Khan</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Russia</td>
<td></td>
</tr>
<tr>
<td>nikolay felodorov</td>
<td></td>
</tr>
<tr>
<td>Djalaliddin Gaibulin</td>
<td>Muzafarbek Inoyatbekov</td>
</tr>
<tr>
<td>Republican Ctr. for Quarantine and Especially Dangerous Infections</td>
<td>Central Asia Inst. of Foot and Mouth Disease</td>
</tr>
<tr>
<td>Shahana Kazmi</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Olga Gavrilova</td>
<td>Babaxab Ismixonov</td>
</tr>
<tr>
<td>RCQEDI, MOH</td>
<td>Kurdemir Regional Veterinary Services</td>
</tr>
<tr>
<td>Muhammad Akber Khan</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Mike Giorgadze</td>
<td>Henry Jahja</td>
</tr>
<tr>
<td>US CRDF</td>
<td>Indonesia</td>
</tr>
<tr>
<td>Angela Kolesnikova</td>
<td>US CRDF</td>
</tr>
<tr>
<td>Vitaly Grashkin</td>
<td>Nurlan Japaraliev</td>
</tr>
<tr>
<td>Saratov Regional Skin Disease Ctr.</td>
<td>Kriilvp, moH</td>
</tr>
<tr>
<td>Russia</td>
<td></td>
</tr>
<tr>
<td>Mubashir A. Khan</td>
<td>Pakistan Medical Research Council</td>
</tr>
<tr>
<td>Mohsin Haider</td>
<td>Anan Jongjeewattana</td>
</tr>
<tr>
<td>DESTO</td>
<td>BIOTEC Central Research Unit</td>
</tr>
<tr>
<td>Pakistan</td>
<td></td>
</tr>
<tr>
<td>Shahana Kazmi</td>
<td>Pakistan</td>
</tr>
<tr>
<td>Vitaly Grashkin</td>
<td></td>
</tr>
<tr>
<td>weitly Grashkin</td>
<td></td>
</tr>
<tr>
<td>Alex Hall</td>
<td></td>
</tr>
<tr>
<td>Gainesville, FL</td>
<td></td>
</tr>
<tr>
<td>Kasia Kazymbekova</td>
<td>Pakistan</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Organization/Institution</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Alexander Konchin</td>
<td>RIHTOP</td>
</tr>
<tr>
<td>Prasad Kuduvalli</td>
<td>Impart</td>
</tr>
<tr>
<td>David Kuprava</td>
<td>Biokombinat</td>
</tr>
<tr>
<td>Natalya Kurkova</td>
<td>Golitsino</td>
</tr>
<tr>
<td>Olexander Kustan</td>
<td>Kharkiv IECVM</td>
</tr>
<tr>
<td>Elena Kuznetsova</td>
<td>RIHTOP</td>
</tr>
<tr>
<td>Jim LeDuc</td>
<td>US CRDF</td>
</tr>
<tr>
<td>Keane Leitch</td>
<td>Cornell Univ.</td>
</tr>
<tr>
<td>Irene Lerman</td>
<td>US CRDF</td>
</tr>
<tr>
<td>Dmitriy Losovoi</td>
<td>Pokrov</td>
</tr>
<tr>
<td>Eleanor Low</td>
<td>Univ. of Hawaii</td>
</tr>
<tr>
<td>Socorro Lupisan</td>
<td>Research Inst. of Tropical Medicine</td>
</tr>
<tr>
<td>Alena Magay</td>
<td>Republican Sanitary Epidemiological Service</td>
</tr>
<tr>
<td>Nadia Nostafa Mahmoud</td>
<td>Central Health Lab.</td>
</tr>
<tr>
<td>R. K. Mainylovk</td>
<td>Karakol Region Branch of RCQEDI</td>
</tr>
<tr>
<td>Irma Makalinaro</td>
<td>Univ. of the Philippines</td>
</tr>
<tr>
<td>Dominik Mayr</td>
<td>Abedd Lab &amp; Vet Service, Ltd.</td>
</tr>
<tr>
<td>Jo Ann Medina</td>
<td>DoorAdo Equipment Specialties, Corp.</td>
</tr>
<tr>
<td>Gedim Meherremov</td>
<td>MOAG, State Veterinary Service</td>
</tr>
<tr>
<td>Nena Moonier</td>
<td>Argonne National Lab.</td>
</tr>
<tr>
<td>Calcita M. Morales</td>
<td>Bureau of Animal Industry</td>
</tr>
<tr>
<td>Megan Morgan</td>
<td>NIH/NBBTP</td>
</tr>
<tr>
<td>Ramazon Murodov</td>
<td>Republican Anti-Plague Station</td>
</tr>
<tr>
<td>Ahmad Nadeem</td>
<td>DESTO</td>
</tr>
<tr>
<td>Maya Nair</td>
<td>Univ. of South Texas Health Science Ctr.</td>
</tr>
<tr>
<td>Abdulaziz Y. Najmaddin</td>
<td>High Inst. of Health Sciences</td>
</tr>
<tr>
<td>Cynthia M. Nalo-Ochona</td>
<td>Bureau of Animal Industry</td>
</tr>
<tr>
<td>Natalia Negriy</td>
<td>Serpukhov</td>
</tr>
<tr>
<td>Lybov Nekrasova</td>
<td>SES</td>
</tr>
<tr>
<td>Leila Macedo Oda</td>
<td>ANBio</td>
</tr>
<tr>
<td>Nikolay Ogenv</td>
<td>Vladmir</td>
</tr>
<tr>
<td>Tinatin Onashvili</td>
<td>Lab. of Ministry of Agriculture</td>
</tr>
<tr>
<td>Abuzer Orugov</td>
<td>Republican Vet. Lab</td>
</tr>
<tr>
<td>Gopal Pande</td>
<td>Centre for Cellulae and Molecular Biology</td>
</tr>
<tr>
<td>Luxi Riajuni Pasaribu</td>
<td>Litbangkes</td>
</tr>
<tr>
<td>Dilip Rewa Patil</td>
<td>National Inst. of Virology</td>
</tr>
<tr>
<td>Natalya Pavlovich</td>
<td>Rostov API</td>
</tr>
<tr>
<td>Oгла Podladchikova</td>
<td>Rostov API</td>
</tr>
<tr>
<td>Izabela Puskarz</td>
<td>BioRelix, Inc.</td>
</tr>
<tr>
<td>Anak Agung Gde Putra</td>
<td>DIC</td>
</tr>
<tr>
<td>Huma Quershi</td>
<td>Pakistan Medical Research Council</td>
</tr>
<tr>
<td>Abdul Rashid</td>
<td>PARC</td>
</tr>
</tbody>
</table>
New Corporate Memberships

Air Systems Technologies, Inc.
Avon, MA
James Sigler

Asepsis Air Control
Issaquah, WA
Tony Jagow
Vancouver, WA
Richard Sullivan

Medical Research Council
London, England
Paul Jackett
Cambridge, United Kingdom
Karen Sewell

Progressive Recovery, Inc.
Dupo, IL
Wayne Humphrey
Shanon Jones
Danile Marks

The Aaron Diamond AIDS Research Ctr.
New York, NY
Mark Muesing
Vincent Sahi
Stephanie Sozomenu

New Members of Existing Corporations

Det Norske Veritas
Antwerp, Belgium
Evelyn Vancauwenbergh

Eisai Research Inst.
Andover, MA
Donna Kolber-Simond
Lana Parent

ESCO Micro PTE, Ltd.
Singapore
Alex Atmadi
Mike Martin

Gen-Probe
San Diego, CA
Jeff Conery
Michelle Kercado

MedImmune, Inc.
Bensalem, PA
David Welsh, III

U.S. Army Inspector General Agency
Arlington, VA
Gary Graves
Paul Leykamm

New Student Member

Miranda Vata
Rutgers Univ.
East Brunswick, NJ

Laboratory Biosecurity Training

Laboratory Biosecurity Training Available on CDC web site at:
www.cdc.gov/dohsbiosecurity_trainingindex.html

This training describes key principles for securing biological agents in research laboratories and biomedical facilities where loss, theft, release or intentional misuse of the agent might have significant public health or economic consequences. After completing this training you should be able to:

- Describe the basis for laboratory biosecurity planning for microbiological laboratories.
- Differentiate between biosafety and biosecurity.
- Describe how to conduct a risk assessment.
- Describe the components of a biosecurity plan.

Successful biosecurity programs should have full support from all levels of management, be site-specific, and be based on an understanding of facility assets and needs. Identifying which agents and infrastructure need to be protected is an individual organization’s management decision. Biosecurity programs should apply corresponding security measures by using a graded approach to reduce risk to an acceptable level.
Calendar of Events

April 28—May 2, 2008
The National Biosafety and Biocontainment Training Program (NBBTP) at Kansas State University
Biosecurity Research Institute, Manhattan, Kansas
Contact: Phone: 678-781-5241; Fax: 678-781-5242; E-mail: info@nbbtp.org; www.nbbtp.org

June 23-26, 2008
American Biological Safety Association (ABSA) Summer Seminar Series and Review Course
Sheraton Inner Harbor Hotel, Baltimore, Maryland
Contact: Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

October 19-22, 2008
American Biological Safety Association (ABSA) 51st Annual Conference
John Ascuaga’s Nugget, Reno, Nevada
Contact: Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

November 9-13, 2008
American Association for Laboratory Animal Science (AALAS) 59th National Meeting
Indianapolis, Indiana
Contact: http://nationalmeeting.aalas.org/future_sites.asp

October 18-21, 2009
American Biological Safety Association (ABSA) 52nd Annual Conference
Hyatt Regency Miami, Miami, Florida
Contact: Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

November 8-12, 2009
American Association for Laboratory Animal Science (AALAS) 60th National Meeting
Denver, Colorado
Contact: http://nationalmeeting.aalas.org/future_sites.asp

October 3-6, 2010
American Biological Safety Association (ABSA) 53rd Annual Conference
Hyatt Regency Denver at Colorado Convention Center, Denver, Colorado
Contact: Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

October 10-14, 2010
American Association for Laboratory Animal Science (AALAS) 61st National Meeting
Atlanta, Georgia
Contact: http://nationalmeeting.aalas.org/future_sites.asp

October 2-6, 2011
American Association for Laboratory Animal Science (AALAS) 62nd National Meeting
San Diego, California
Contact: http://nationalmeeting.aalas.org/future_sites.asp

October 30—November 2, 2011
American Biological Safety Association (ABSA) 54th Annual Conference
Anaheim Marriott Hotel, Anaheim, California
Contact: Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org
The TurnKey Conference 2008 is two days of cutting-edge presentations, courses, and exhibitions… in one “can’t miss” event! A full roster of speakers—all experts in their fields—provides you with the facts you need to make informed decisions every step of the way.

ALN Magazine™ is committed to providing conference attendees with the highest quality speakers and presentations possible. Speakers will provide knowledgeable presentations that include the latest trends, technology, and ideas.

REGISTER ONLINE AT
www.turnkeyconference.com
“Leadership is the art of getting someone else to do something you want done because he wants to do it.”

- Dwight Eisenhower

Atlanta, GA
April 7 - 9, 2008

For more information on this event and other biosafety training programs visit
www.sph.emory.edu/CPHPR/biosafetytraining
Minimize your exposure to risk with the quickest, most effective DECONTAMINATION method available

SAFETY:
- Shortest cycle so lowest risk
- Lower concentrations so less risk
- No Residuals
- Non-carcinogenic

SPEED:
- Quicker cycles means safety (less chance for exposure)

EFFECTIVENESS:
- EPA Registered means absolute decontamination against organisms in a non-ideal setting
- TRUE GAS means better penetration, including HEPA filters
- TRUE GAS means better distribution (no special nozzles required)
- Concentration monitoring means cycle validation and quality assurance

COST:
- Most economical since less equipment is required
- Minimal operational downtime

Gaseous Chlorine Dioxide Systems and Decontamination Services from ClorDiSys Solutions Inc

SAFETY THROUGH SPEED

Features, benefits, and equipment can be seen on our website.

Tel: (908) 236-4100 ClorDiSys Solutions, Inc www.clordisys.com

---

When clean air solutions are important to you, depend on Esco laboratory and cleanroom equipment for proven safety and performance.

We’ll pass your test, too.

Esco offers complete selection of innovation laminar flow, air filtration and cleanroom products for laboratory, pharmaceutical, biotechnology, life science and industrial applications. Choose from basic to sophisticated microprocessor-controlled cabinets, all designed to meet the most demanding requirements in the world. Contact Esco for product information and support. Visit www.us.escoglobal.com.

---

ESCO

WORLD CLASS. WORLDWIDE.

Esco Technologies Inc. • 2940 Turnpike Drive, Suite 15-16 • Hatboro, PA 19040, USA
Tel: 1-800-331-0440 • Fax: 215-851-9661 • E-mail: info@escoglobal.com
us.escoglobal.com • us@escoglobal.com

Esco Micro Pte. Ltd. • 21 Changi South Street 1 • Singapore 486 777
Tel: +65-9542 3633 • Fax: +65-9542 9329 • esco@escoglobal.com
www.escoglobal.com
• Four Days of Pre-Conference Courses

• State of the Art Papers and Panels

• Exhibits Showcasing the Latest in Laboratory Technology

• Opportunities to Network with Your Peers

51st Annual Biological Safety Conference

ABSA
American Biological Safety Association

October 19-22, 2008
John Ascuaga's Nugget
Reno/Sparks Nevada