Select Agent Program and Biosafety Improvement Act of 2009 (Introduced in Senate)

111th CONGRESS
1st Session
S. 485

To reauthorize the Select Agent Program by amending the Public Health Service Act and the Agricultural Bioterrorism Protection Act of 2002 and to improve oversight of high containment laboratories.

IN THE SENATE OF THE UNITED STATES
February 26, 2009

Sec. 202 Improvement of Training for Laboratory Personnel
• Requires HHS and USDA to work with professional associations and international health organizations to develop minimum standards for biosafety and biosecurity training for BSL 3 and 4 lab personnel.

Sec. 203 Biological Laboratory Incident Reporting System
• Requires HHS and USDA to establish a voluntary Biological Laboratory Incident Reporting System, through which lab personnel can report biosafety and biosecurity incidents of concern. HHS and USDA could also identify trends in such incidents and protocols for biosafety and biosecurity improvements.
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Guest Editorial

The American Research University and Institutional Biosafety Committees
Kenneth I. Shine and Richard St. Onge .............................................................. 119

Articles

The Efficiency of HEPA Filters in the Air-handling System of a Bio-containment Laboratory in India
Shivchandra C. Dubey, Harshad V. Murugkar, Ramesh K. Kaushik, and Diwakar D. Kulkarni ............... 121

Large-scale Inactivation of Bacillus anthracis Ames, Vollum, and Sterne Spores Using Vaporous Hydrogen Peroxide
James V. Rogers, William R. Richter, Morgan Q. Shaw, and Adrienne M. Shesky .................................. 127


Consequences of Failure to Apply International Standards for Laboratory Biosafety and Biosecurity: The 2007 Foot-and-Mouth Disease Outbreak in the UK
Catherine Rhodes ................................................................................................. 144

Special Features

Molecular Biosafety—Safety Overview of Techniques Involving miRNAs, siRNAs, and Other Small Regulatory RNAs
Margy S. Lambert ............................................................................................... 150

Ask the Experts—Protecting Surfaces in BSL-3 Suites and Animal Facilities—Are Work Surface Pads and Disinfectant or Sticky Floor Mats the Answer?
John H. Keene ..................................................................................................... 153

About the Cover

The Select Agent Program and Biosafety Improvement Act of 2009 (S.485) introduced by Senators Burr and Kennedy provides suggestions for improving biosafety, reauthorizes funding for the Select Agent Program and recommends a review of that program and associated topics (http://thomas.loc.gov/cgi-bin/query/z?c111:S.485:). Two proposed improvements to biosafety have been summarized on the cover. A survey of biosafety professionals in the U.S. was conducted by Chamberlain et al. The survey focused in part on biosafety training and incident reporting practices, and provides insights into strengthening biosafety programs. For more information on this timely topic, please see “Biosafety Training and Incident-reporting Practices in the United States: A 2008 Survey of Biosafety Professionals” on pages 135-143.
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The American Research University and Institutional Biosafety Committees

Kenneth I. Shine and Richard St. Onge
The University of Texas System, Austin, Texas

On November 17, 1944, President Franklin D. Roosevelt sent to the Office of Scientific Research and Development, directed by Vannevar Bush, a series of questions about the future of scientific research and development after World War II. Among the questions he asked was, “With particular reference to the war of science against disease, what can be done now to organize a program for continuing in the future the work which has been done in medicine and related sciences?” President Roosevelt observed, “New frontiers of the mind are before us, and if they are pioneered with the same vision, boldness, and drive with which we have waged this war, we can create a fuller and more fruitful employment and a fuller and more fruitful life.” In July 1945, Vannevar Bush responded to the President’s request with a report entitled Science: The Endless Frontier. Among the very important recommendations made by Vannevar Bush was the identification that basic or fundamental research should be carried out in America’s universities, while industry would focus on applied research and development. This reliance upon the university accelerated a trajectory that made American universities the leading research universities in the world.

Unlike research institutes in Western Europe or the focus of research in industry in Japan, the American university combined education and training with research. Many of the most important ideas in biomedical research come from young PhD, MD, and post-doctoral students. These same students are the workhorses of the research enterprise.

Combining research and education was a master stroke which brought students to America from all over the world. In contrast to other countries, the United States developed a peer review process, in which the best ideas were funded by agencies such as the National Institutes of Health (NIH) and the National Science Foundation, rather than the European practice of providing money to senior professors who then directed the research. An essential feature of this method in the United States was the decentralization of the research enterprise, fostering a diversity of approaches, innovative ideas, and environments enriched by the interaction between the researchers and their students.

A central feature of this decentralization was the trust placed in the University to oversee the actual conduct of research. The university and its investigators were trusted to use monies granted from the federal government to carry out research in an appropriate and cost-effective manner. Investigators were trusted to record scientific data and to report it accurately and appropriately in scientific journals. The peer review process used for grants became a central feature in the determination of publication. The institution was also trusted to organize institutional review boards to assess the ethical and scientific appropriateness of human participation in scientific research. The system was by no means flawless and from time to time significant problems arose regarding informed consent, conflict of interest, and some unsafe practices. However, the solution applied to these challenges again was based upon a level of trust attested through accreditation processes, which themselves were conducted by scientific and ethical peers. A similar approach was taken for the use of animals in research, with the creation of institutional animal care committees and an accreditation process.

Institutional biosafety committees (IBCs) came into being when scientists and the public, including policymakers, were challenged by the emergence of recombinant DNA as a potential therapeutic device. Although some aspects of unapproved DNA applications to humans were forbidden by law and in some cases involved criminal penalties, the overall oversight for DNA research was through the trust placed in IBCs. More recently, the IBCs have been responsible for even broader issues of safety including select agents and other biological and chemical risks on the campus. As with other aspects of this endeavor, the system has not always been perfect and, particularly as a consequence of whistle-blowers, deviations from policy have been identified and have led to sanctions by agencies of the federal government. Although examples of scientific misconduct have been identified, they still remain relatively rare events given the size and scope of the scientific undertaking. In general, however, the system of trust placed in the University, its scientists, and its committees has been remarkably successful.

In the spirit of this delegated responsibility of trust, The University of Texas System (UT System) organized, in
Guest Editorial

collaboration with the NIH, an educational conference on the role of the IBCs in the UT System entitled, “IBC 101.” This conference, held August 18-19, 2005, was originally designed for UT System institutions, but over 30 institutions from 12 states attended the meeting. It was not only instructive but also led to the development of a template which IBCs could use as a self-assessment tool to evaluate their own performance, as well as for training and education. Participants found the experience so useful that a subsequent conference, “IBC 201,” was held in September 2008, in collaboration with the NIH, the Centers for Disease Control and Prevention (CDC), and the U.S. Department of Agriculture. At this conference, the template was updated.

The NIH and the CDC convened the first national meeting for IBC participants in June 2009. This meeting brought together over 250 individuals who discussed an intense and comprehensive approach to the challenges posed by the responsibilities of IBCs. As a result of this meeting, the NIH has made available a self-assessment template for IBCs that is, in part, based on the UT System model and further adapted for use across the country.

IBCs continue to be essential components in the maintenance of trust which has made American research universities and other research institutions the best in the world. They deserve greater recognition within their institutions for the role they play, as well as clearer articulation of institutional support at the very highest levels. In many cases they continue to be underresourced in both personnel and financing for the magnitude of the responsibility they must fulfill. In this regard, the NIH has indicated a program of site visits which can be used not only to assess the state of IBC’s effectiveness on campus, but also can be effectively used as an educational tool for institutional leaders to educate them on the importance of properly supported and properly functioning IBCs.

A major challenge and opportunity for IBCs is an enhanced set of interactions with other institutional entities such as the institutional review boards, the institutional animal care and use committee, and other comparable committees. Enhanced communication and coordination among these entities can facilitate the improved operations of each. The involvement of human participants and animals in studies involving select agents or DNA is a joint responsibility of multiple committees which can coordinate these activities more effectively in many cases. At the same time, these entities have major educational responsibilities within the institution. Coordinating these educational activities to make them efficient and effective, particularly for time-pressured investigators and their staff, can be an important contribution to effectiveness and efficiency. We have found the self-assessment tool to be a useful vehicle to understand internal operations.

As a keynote speaker at the 2009 IBC meeting, it was Kenneth Shine’s privilege to salute the dedication and expertise reflected in the participants and in those who work to promote institutional biosafety. It is essential that the scientific community understand the importance of this activity and the trust which society has placed in it. The IBCs have helped to allow American science to operate in the decentralized university-based role that continues to lead to opportunities for individuality, creativity, and local initiative. Preserving trust, through the IBCs and other institutional committees with similar responsibilities, is an essential feature if American science is to prosper and the American research university is to be preserved.

Acknowledgment

The important contributions of Ms. Janet Cole to the organization of IBC 101 and IBC 201 are gratefully acknowledged.

References


The Efficiency of HEPA Filters in the Air-handling System of a Bio-containment Laboratory in India

Shiv C. Dubey, Harshad V. Murugkar, Ramesh K. Kaushik, and Diwakar D. Kulkarni

High Security Animal Disease Laboratory, IVRI, Bhopal, Madhya Pradesh, India

Abstract

The High Security Animal Disease Laboratory (HSADL) in Bhopal, India is a BSL-4 laboratory that was constructed following the Labystad (The Netherlands) model and was commissioned in 1997 as a biocontainment facility to undertake research and diagnostic work on exotic and emerging diseases of animals in the country. The laboratory’s air-handling system is comprised of 23 air-handling units (AHUs) with 97 HEPA filters (Anfilco, India) fitted in 92 filter housings. Preventing environmental contamination is achieved by maintaining graded negative pressure ranging between -50 to -200 Pascals. This paper discusses the efficiency of the HEPA filters following the dioctylphthalate (DOP) testing carried out over a decade-long period. Out of the 81 (83.5%) filters replaced during this period, HEPA filters of 59 (72.83%) housings were replaced once; of 17 housings (20.98%) were replaced twice, and of 6 housings (7.4%) were replaced three times. Major reasons for the replacement of these filters include blockage (48.18%) and lowered efficiency (51.85%) of the filter medium. The majority of the filter changes at HSADL can be attributed to the routine ageing of the filters, resulting in the degraded mechanical strength of the filter medium or choking due to aerosols and dust. Some areas of the laboratory encountered a higher rate of filter change and the reasons thereof are discussed. These observations regarding HEPA filter efficiency in AHUs are probably the first in a tropical environment. The possible roles of factors such as the availability of a dense tree canopy in the direction of the air flow, the close location of the lab to a large water body, and the lab’s distance from the dusty highways in the longevity of this laboratory’s HEPA filters are also discussed. This study re-emphasizes the need for regular monitoring of the HEPA filter function in the AHUs and their prompt replacement when damaged to ensure effective bio-containment.

Introduction

High efficiency particulate air (HEPA) filters are widely used all over the world to prevent contamination of the environment from microbiological containment laboratories and experimental animal establishments. Since the time when HEPA filters were introduced in military establishments for protection against particulate chemical, biological, and radiological warfare agents (First, 1996), HEPA filters have become an integral part of air-handling systems in virtually all laboratory and pharmaceutical establishments where clean and safe air is of paramount importance. These filters have a minimum efficiency of 99.97% for particles ≥0.3 microns in diameter and are the principal components of any air-handling system (AHS) in bio-containment laboratories (Abraham et al., 1998). One of the major challenges and requirements faced by bio-containment laboratories has been to ensure full functional capacity of the HEPA filters. The HEPA filters are comprised of five major components: (1) extensively pleated filter medium; (2) corrugated separators, usually made of aluminum; (3) a frame that holds the filter medium and separators; (4) adhesive that cements the filter and separators to the frame; and (5) a gasket to make the filter airtight. In an extensive review on filter ageing, First (1996) has reported that in the due course of time HEPA filters undergo mechanical damage or ageing wherein one or more filter components are subjected to wear, embrittlement, corrosion, and other physical and chemical changes. These changes ultimately result in the failure of the integrity and efficiency of the HEPA filters. Since the AHS of the containment laboratories is required to be functional around the clock and throughout the year, the integrity and efficiency of HEPA filters must be checked at frequent intervals as an integral part of the biosafety protocol of the bio-containment facility (Abraham & McCabe, 2007).

Failure-proof functioning of HEPA filters in air-handling systems is a prerequisite for any biocontainment laboratory, and the assessment of these filters’ reliability over a period of years plays an important role in ensuring higher bio-containment standards in a biological laboratory (Abraham et al., 1998). The importance of evaluating HEPA filter efficiency in containment laboratories handling exotic pathogens for a particular country or those with a higher risk of airborne transmission cannot be overemphasized. HSADL in Bhopal, India is a BSL-4 laboratory established to undertake...
research and diagnostic work on the exotic and emerging diseases of livestock in India. The air exchange to the entire containment area is carried out through a complex air-handling system through individual air-handling units (AHUs). This paper analyzes the performance of HEPA filters used in the air-handling system for over a decade.

**Materials and Methods**

The containment area of the entire laboratory is divided into a laboratory (1,875 m$^2$), an animal wing (1,135 m$^2$) located at the ground level and an effluent treatment plant (ETP) (2,840 m$^2$) located at 3 m or 5.3 m below ground level. Preventing environmental contamination by aerosols is achieved by maintaining negative pressure in the containment area between -50 to -200 Pascals. To ensure the maintenance of this negative pressure inside the containment area, the electrical interlocking system controls the supply and exhaust air in such a way that the supply fan starts operating along with the initiation of the operation of the exhaust fan. The laboratory is supplied with a dedicated uninterrupted electrical supply; however, in the case of an accidental electric failure negative pressure is maintained with the help of zero leakage dampers and the flow control dampers that regulate the air supply and exhaust.

The air-handling system of the entire laboratory area is administered with the help of 23 air-handling units (AHUs). Depending on the size of the unit, and the air exchange requirement based on the functions of individual laboratory unit, these 23 AHUs (Caryaire Equipments India Pvt Ltd) are compartmentalized into 92 filter housings containing 97 HEPA filters (Anfilco, India). The air supply to the laboratory is carried out via a pre-filter with an efficiency of 99% down to 5 microns. The filtering media of this pre-filter is made up of a synthetic polymer and is supported by aluminum-anodized mesh. The filter pack is sealed to the frame by an epoxy-based adhesive. It has a rated flow of 2,000 CFM, having the initial pressure drop and final pressure drop of 6.5 mm WG and 19.0 mm WG, respectively. In addition to this pre-filter, a filter change is recommended. In addition, each filter housing is fitted with a differential pressure meter (DPM) that measures the difference in the air pressure before the air enters the pre-filter and when the air exits the HEPA filter. An increase in the differential pressure above 50 mm WG indicates a blocking of the filters and requires immediate replacement.

For the sake of data analysis, the period from 1st April to 31st March is taken as 1 year. The installation of the air-handling unit for the laboratory and its commissioning were completed in November 1997; therefore, the data are analyzed for a period of 10 years (i.e., April 1998 to March 2008). Details regarding filter locations, negative pressure maintained in these areas, the air flow inside the laboratory areas etc. are given in Table 1.

**Results and Discussion**

The data analyzed for the period of April 1998 to March 2008 have indicated that a total of 81 filters were replaced during the 10 years after commissioning. The details are presented in Table 2. During installation of the HEPA filters, three filters were replaced immediately after their installation since leakage was observed during final testing after installation and prior to commissioning of the laboratory. These replacements were carried out due to damage to the filters during their installation and hence have not been accounted for in Table 2. After the commissioning of the laboratory, out of the total 81 HEPA filters replaced, 59 filters (60.82%) were replaced once. Seventeen filters among these previously replaced filters showed filter damage and had to be re-replaced, whereas five filters (of second change category) were again replaced following failed filter efficiency tests.

In the present study, the reasons for filter replacement are attributed to lowered efficiency of the filter medium and blockage of the filter medium.

Owing to the reduced efficiency of filter medium below the recommended level (less than 99.97%); a total of 33 filters (34.02%) had to be replaced. The filters’ low efficiency could have occurred because the filters lost mechanical strength, as is known to happen
with ageing filters (Edwards, 2002), or because of the failure of the epoxy seal holding the filter medium to the filter frame (Abraham & McCabe, 2007). Abraham et al. (1998), due to their experience with HEPA filter replacement in a biological laboratory, have reported that the lower efficiency of the filter medium occurs because of leakages (usually “pinhole” leaks) or defects in the gasket seal securing the filter frame to its housing. Since no specific study was carried out in this regard, the possibility for either or both of these reasons for the efficiency failure cannot be ruled out. In summer, the ambient temperature of Bhopal city is in the range of 40°C - 45°C for at least 2 months. Since the AHUs are located on the top floor that lacks any cooling system, such high temperatures could also result in some damage to the filter’s polymer or epoxy seal and could have resulted in reduced filter efficiency.

In any high-containment microbiological laboratory, HEPA filters perform the basic function of biocontainment through the air-handling systems associated with microbiologically secure areas to prevent the escape of infectious aerosols. A high differential pressure meter (DPM) value indicates the blocking of the filters. In the present case, 26 filters (26.8%) had to be changed due to blocking. In the majority of the housings (except the ones in laboratory animal rooms, which are fitted with double HEPA filters), the pre-filters preceded HEPA filters during air supply and prevented HEPA filter blocking due to larger particles (> 5µ); however, replacement of more than 26% of the filters due to blocking indicates the involvement of smaller particles (3µ - 5µ) in blocking the filters. The filter blockage could also be due to a reduction in the binding compound content in the filter medium (Edwards, 2002), consequently causing more frequent blocking of the filters present in the air exhaust system, or physical medium failure (First, 1996). Other laboratories similar to this one have also experienced these problems of filter leakages and filter blocking due to dust loading (Abraham et al., 1998; Edwards, 2002).

Analysis of filter changes based on location is presented in Table 3. It is commonly observed that HEPA filters from the areas with minimum movement have more longevity. The filters for fume hoods and all airlocks are still functioning after 10 years without any damage or blockage, whereas 16 filters (11+4*+1** of 18 filter housings) from Central Laboratory needed to be replaced.

One interesting finding in the study was the high

### Table 1
Details of AHU Data at HSADL, Bhopal

<table>
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<tr>
<th>Filter Location</th>
<th>Air Pressure (Pascals)</th>
<th>Air Flow (CFM)</th>
<th>Number of Filter Housings</th>
<th>Number of Filters</th>
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<td>-100</td>
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<td>Central Laboratory</td>
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<td>12440</td>
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<td>810</td>
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<td>-120</td>
<td>4650</td>
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<td>Workshop &amp; BSO Office</td>
<td>-120</td>
<td>3790</td>
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<td>Central Lab Fume Hood</td>
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<td>705</td>
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<td>Radioisotope Lab</td>
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<td>3023</td>
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<td>2100</td>
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<tr>
<td>Effluent Treatment Plant (ETP) Basement (-3 m)</td>
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</tr>
<tr>
<td>Animal Corridor</td>
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<td>Combined Room</td>
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<td>Basement Airlock (-3 m)</td>
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<tr>
<td>Bio-Cooker Room (Rendering Plant) (-5.3 m)</td>
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<td><strong>Total</strong></td>
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<td></td>
<td><strong>92</strong></td>
<td><strong>97</strong></td>
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*4*: Central Laboratory - 18 filters
**1**: Central Laboratory - 18 filters
frequency of filter replacement in the effluent treatment plant (Table 3), where all six filters from six housings were replaced, five were replaced for a second time, and four for a third time. Five out of six failures were due to filter blockage. Of these, three filters were replaced three times and five were replaced twice, indicating a higher replacement rate for this AHU compared to the others in the laboratory. The effluent treatment plant has steam autoclaves and the excess steam released from the valves to maintain steam pressure during autoclaving results in an exceptionally high moisture content and higher aerosolization of the ETP environment. Abraham et al. (1998), in their study on HEPA filter replacement in a biological laboratory, have attributed higher deterioration of filters due to water, dust, and smoke and have reported higher filter replacement rates where condensation of water vapor in vent filters resulted in filter damage. The ETP is located in the basement with three-quarters of it at 3 m below ground level and carries the ducting and piping from every part of the lab. Accumulated dust on these parts is difficult to clean, so dusting of this area is carried out manually by sweeping and could result in greater filter blockage. A change in cleaning practices using motorized vacuum cleaners is being recommended for the laboratory, and the impact of this change in practice could be the subject of future studies.

Following commissioning of the laboratory in 1997, the first filter replacement was carried out in 2002, and

<table>
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<th>Year</th>
<th>Number Changed Due to DOP Test Failure</th>
<th>Number Changed Due to Filter Blockage</th>
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<td></td>
<td>1st change</td>
<td>2nd change</td>
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<td>3/97 (3.09)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1999-00</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2000-01</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2001-02</td>
<td>7/94 (7.45)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2002-03</td>
<td>0</td>
<td>1/15 (6.67)</td>
<td>0</td>
</tr>
<tr>
<td>2003-04</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2004-05</td>
<td>11/82 (13.41)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2005-06</td>
<td>2/63 (3.17)</td>
<td>3/37 (8.11)</td>
<td>0</td>
</tr>
<tr>
<td>2006-07</td>
<td>7/57 (12.28)</td>
<td>2/42 (4.76)</td>
<td>0</td>
</tr>
<tr>
<td>2007-08</td>
<td>3/49 (6.12)</td>
<td>3/51 (5.88)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>33/97 (34.02)</td>
<td>9/59 (15.25)</td>
<td>0</td>
</tr>
</tbody>
</table>

Explanation:
- Denominator in columns 2 and 5 indicates total number of unchanged filters, whereas numerator indicates number of filters changed out of the total available up to that year (cumulative data).
- For second change, denominator in columns 3 and 6 indicates number of filters available from only previously changed (first change from columns 2 and 5, respectively), whereas numerator indicates number of filters changed out of the total available up to that year (cumulative data).
- For third change, denominator in columns 4 and 7 indicates number of filters available from only previously changed (second change from columns 3 and 6), whereas numerator indicates number of filters changed out of the total available up to that year (cumulative data).
- Data in columns 8, 9, and 10 indicates total number of filters changed due to DOP test failure and blockages.
- Figures in parentheses indicate percentage.
the majority of the filters (i.e., 79%) were replaced 6 years after laboratory commissioning. The longevity of the HEPA filters depends on a number of factors, such as dust concentration, temperature, humidity, severe conditions like earthquake or violent explosion resulting in a long duration of pressure pulses or shock pulses (Osaki & Kanagawa, 1989), air supply and exhaust pressure, quality of the filter medium, or sealant system-induced pressure pulses (e.g., sudden starting of air blowers). In this period, HSADL has not experienced any adverse climatic changes, such as high-speed winds, dust storms, etc., that could result in large-scale filter damage. As suggested by Edwards (2002), the only significant pressure pulse that could be experienced by HEPA filters in a biological laboratory could happen when AHS fans start or fan swap occurs between an in-service fan and stand-by fan. Some filter damage due to such procedures might have occurred at HSADL too. Considering the dry and dusty tropical environment of the region for most of the year, one would expect a remarkably reduced shelf life for the HEPA filters in such laboratories. Longer filter shelf life in this study could be attributed to many probable reasons including the following: (1) The laboratory is located in very close proximity to a lake that brings northern winds with a relatively lower concentration of dust in the direction of the laboratory; (2) The laboratory is situated about 1 km from the highway; consequently, the dust concentration is relatively low as compared to many other places in the region; (3) The laboratory campus has a fairly thick tree canopy that might have trapped a good amount of the dust.

The majority of the filter changes currently at HSADL can be attributed to the routine ageing of the filters resulting in the degraded mechanical strength of the filter medium or choking due to aerosols and dust. Replacing all the filters of the same age when degradation of some of the filters is observed in a laboratory has been suggested (Edwards, 2002). However, this decision needs to be based on laboratory-specific requirements, logistics, the economics of filter-changing, and most importantly the availability of a foolproof monitoring mechanism to check the quality of the filters on a regular basis.

Table 3
Area Distribution of HEPA Filters and Their Replacements

<table>
<thead>
<tr>
<th>Area of Laboratory</th>
<th>HEPA Filters Available</th>
<th>Changed due to DOP Test Failure</th>
<th>Changed due to Blockage</th>
<th>Total Number of Filters Replaced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Corridor</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Inner Change Room</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Laboratory</td>
<td>18</td>
<td>5+3*</td>
<td>6+1*+1**</td>
<td>11+4*+1**</td>
</tr>
<tr>
<td>Dirty Dispatch</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>7</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Cell Culture</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workshop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSO Office</td>
<td>6</td>
<td>3+2*</td>
<td>2</td>
<td>5+2*</td>
</tr>
<tr>
<td>Central Lab Fumehood</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Radioisotope Lab</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Glassware Wash, Autoclave Room</td>
<td>4</td>
<td>4+1*</td>
<td>0</td>
<td>4+1*</td>
</tr>
<tr>
<td>Effluent Treatment Plant</td>
<td>6</td>
<td>1+1*</td>
<td>5+4*+4**</td>
<td>6+5*+4**</td>
</tr>
<tr>
<td>Animal Corridor</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lab Animal Room</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Post Mortem Room</td>
<td>3</td>
<td>2+1*</td>
<td>0</td>
<td>2+1*</td>
</tr>
<tr>
<td>Isolator Room</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Large Animal Rooms</td>
<td>8</td>
<td>1</td>
<td>3+3*</td>
<td>4+3*</td>
</tr>
<tr>
<td>Small Animal Rooms</td>
<td>4</td>
<td>0</td>
<td>3+1*</td>
<td>3+1*</td>
</tr>
<tr>
<td>Animal Airlock</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basement Airlock</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Laboratory Airlock</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cooker Room</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>59+17*+5**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Second Change
** Third Change
Acknowledgements

The authors are grateful to the Indian Council of Agricultural Research, New Delhi, and the Directors of the Indian Veterinary Research Institute for their constant support in the operation and maintenance of bio-containment facilities. The authors are equally indebted to former Joint Directors of HSADL, Dr. G. C. Mohanty and Dr. H. K. Pradhan, for their constant technical advice. Help rendered by Mr. Asanna Badge and Mr. Kedar Sharma, site in-charge, Suvidha Engineers, for their assistance in compiling the data is also appreciated.

References


Erratum: Cover Art in Applied Biosafety (Volume 14, Number 2, 2009)

An error in file reading has resulted in a misrepresentation of the geographical locations of biosafety organizations on the cover art published in Volume 14, Number 2, 2009. A corrected map is provided in this erratum. The information contained in the “About the Cover” description is accurate and serves to assist in locating the biosafety organizations on the map. The stars represent the locations of the following global biological safety associations and working groups: African Biosafety Association, American Biological Safety Association, ABSA-Canada, Asia-Pacific Biosafety Association, Biosafety Association of Central Asia and the Caucuses, Biosafety Association of Pakistan, European Biosafety Association, Israeli Biological Safety Association, Japanese Biosafety Association, National Biosafety Association of Brazil, Philippines Biosafety and Biosecurity Association, Taiwan Biological Safety Association, and the Thailand Biosafety Working Group.
Large-scale Inactivation of *Bacillus anthracis* Ames, Vollum, and Sterne Spores Using Vaporous Hydrogen Peroxide

James V. Rogers, William R. Richter, Morgan Q. Shaw, and Adrienne M. Shesky

Battelle Memorial Institute, Columbus, Ohio

**Abstract**

This study evaluated the inactivation of *Bacillus anthracis* Ames, Vollum, and Sterne spores on various materials (glass, Hypalon® rubber glove, and stainless steel) using vaporous hydrogen peroxide fumigation of a ~15 m² aerosol research and component assessment (ARCA) chamber. Suspensions of each spore type (~1 x 10⁸ CFU) were dried on coupons made from each type of test surface and exposed to vaporous hydrogen peroxide fumigation for a decontamination time of 5.5 hours. For these three materials, the log reductions ranged from 7.7 to 7.9, 8.2 to 8.5, and 7.6 to 7.8 for *B. anthracis* Ames, Vollum, or Sterne spores, respectively. The effectiveness of vaporous hydrogen peroxide fumigation on the growth of *Geobacillus stearothermophilus* biological indicators (BI) was evaluated in parallel as a qualitative assessment of decontamination. At 1 and 7 days post-exposure, all decontaminated BI exhibited no growth. This study provides information for using vaporous hydrogen peroxide fumigation as an approach for the surface decontamination of *B. anthracis* spores within a large-scale chamber.

**Introduction**

Fumigants (e.g., gases or vapors) for decontaminating *Bacillus anthracis* spores have been utilized for decades to treat animal hides and skins and to mitigate the health problems associated with the handling of contaminated wool and hair (Rogers et al., 2008a). Many of these fumigants have included gaseous or vaporous forms of ammonia, bromine, carbon tetrachloride, chlorine, chlorine dioxide, ethylene oxide, formaldehyde, hydrochloric acid, hydrogen peroxide, iodine, methyl bromide, nitrogen dioxide, ozone, phosgene, sulfur dioxide, and trichloroethylene (Rogers et al., 2008a). Fumigants offer advantages over liquids, sprays, and wipes as they can be easily dispersed within an extensive volume (e.g., rooms or buildings) and provide coverage over the corresponding large surface areas. More recently, some of these fumigants have been tested and implemented following the release of *B. anthracis* spores in the mail in 2001. Specifically, hydrogen peroxide, chlorine dioxide, and formaldehyde were used to decontaminate five mail facilities and two office buildings (Canter, 2005; Canter et al., 2005).

The use of vaporous hydrogen peroxide is a common approach for decontamination and disinfection in the scientific, medical, and pharmaceutical fields. Hydrogen peroxide is considered less toxic to humans than other fumigants (e.g., formaldehyde, chlorine dioxide); therefore, it has been widely used to treat laboratory and medical equipment, pharmaceutical facilities, hospital rooms, ambulances, animal holding rooms, and air ducts (Anderson et al., 2006; Dryden et al., 2008; Fichet et al., 2004; French et al., 2004; Heckert et al., 1997; Hillman, 2004; Johnston et al., 2005; Klapes & Vesley, 1990; Krause et al., 2001; Krishna et al., 2000; Verce et al., 2008; Wagenaar & Snijders, 2004). The use of hydrogen peroxide as a fumigant, dry mist, or an aqueous solution promotes decontamination efficacy against a wide range of microorganisms, including bacterial spores, vegetative bacteria, fungi, viruses, and bacteriophages (French et al., 2004; Grare et al., 2008; Hall et al., 2007; Hall et al., 2008; Heckert et al., 1997; Hillman, 2004; Johnston et al., 2005; Klapes & Vesley, 1990; Melley et al., 2002; Otter & Dudde-Niekiel, 2009; Rastogi et al., 2009; Rogers et al., 2005; Rogers et al., 2008b; Rogers & Choi, 2008).

For laboratory testing of fumigant decontamination efficacy against virulent *B. anthracis* spores, studies have been done at the bench scale using small-sized chambers of approximately 300 L (Rogers et al., 2005; Rogers et al., 2007; Rogers et al., 2008a). However, as research keeps moving forward into the testing of fumigants against *B. anthracis* spores and other biological select agents, the need to move towards larger-scale testing is growing. Recently, our facility has acquired a custom-made biosafety level 3 (BSL-3)-capable aerosol research and component assessment (ARCA) chamber that is designed to generate aerosols of biological agents to enable testing of sensors, detectors, and decontaminants, or to enable the ability to perform other large-scale biological agent studies. The ARCA chamber has a total internal working volume of approximately 520 ft³ (~14.7 m³) and working surface area of approximately 58.6 m². During operation, the airflow through the ARCA is unidirectional with complete exhaust from the chamber. As the ARCA chamber is large enough for multiple people to enter into the main chamber, it is crucial that successful decontamination is performed and demonstrated to maximize safety, prevent the risk of any potential secondary aerosolization and exposure, and enable safe removal of any test equipment or devices.
Therefore, the purpose of this study was to demonstrate the ability to decontaminate multiple strains of B. anthracis spores on surfaces within the ARCA chamber.

Materials and Methods

Test Organisms
All portions of testing were performed under BSL-3 conditions. Spores of the virulent B. anthracis Ames strain were prepared by fermentation as previously described (Rogers et al., 2005). Virulent B. anthracis Vollum and avirulent B. anthracis Sterne spores were prepared using a shaker flask method as previously described (Rogers et al., 2006). The purified spore preparations were resuspended in sterile water and evaluated by phase-contrast microscopy. Preparations having >95% refractile spores with <5% cellular debris were enumerated, diluted to approximately 1 x 10^9 colony-forming units (CFU)/ml, and stored at 2º-8ºC.

Test Materials
Three materials commonly found in the ARCA chamber (The Baker Company, Sanford, ME) were used as the test surfaces and included glass, Hypalon® glove, and stainless steel. The glass coupons were plain microscope slides (Fisher Healthcare, Houston, TX), while Hypalon® coupons were cut to approximately 2.0 cm x 2.0 cm from a Hypalon® glove. The stainless steel coupons were approximately 1.5 cm x 6.5 cm. All coupons were sterilized by autoclaving at 121ºC for 20 minutes prior to testing. Visual assessment of the coupons used for the study was performed to ensure that no physical changes or defects such as warping or discoloration occurred as a result of the autoclaving. A visual inspection of the physical integrity and appearance of the test material coupons was also performed before and after decontamination to detect any damage to the test materials.

Decontamination Procedure
Each test coupon was laid flat in a Biological Safety Cabinet (BSC) Class III and inoculated with approximately 1.0 x 10^8 CFU by dispensing ten, 10 µL droplets across each coupon surface as previously described (Rogers et al., 2005). For each type of test material, three coupons were used for decontamination, three coupons were used as controls (inoculated; not decontaminated), and two coupons were used as blanks (not inoculated). Following inoculation, the coupons were allowed to dry overnight, undisturbed. The next day, the inoculated coupons intended for decontamination (and one blank) were transferred into the ARCA chamber. Due to the size and complexity of the ARCA chamber, one of each coupon type (and blanks) were placed in the upstream, middle, and downstream sections (Figure 1) to demonstrate decontamination efficacy throughout the chamber. Four 12-inch fans were placed inside the ARCA chamber at various locations to provide turbulence that would help maximize vaporous hydrogen peroxide distribution (Figure 1). Control coupons (and blanks) were transferred to a Plas Labs Model 830-ABC Compact Glove Box (Plas Labs, Inc., Lansing, MI), and the coupons were placed lying flat, inoculated surface side up on a wire rack.

In parallel, Steris Sporex® single species biological indicators (BI) containing 4 x 10^9 Geobacillus stearothermophilus (ATCC 7593) spores (STERIS Corporation, Mentor, OH) were used to evaluate decontamination. Sixteen BI were placed at separate locations within the ARCA chamber and subjected to decontamination; a single BI affixed to the outside of the ARCA was not decontaminated and used as a positive control (Figure 1). Additionally, VHP Chemical Indicator NB305 strips (STERIS Corporation) were used to provide a visual indication (color change) that fumigation of the ARCA chamber had occurred. Sixteen indicator strips were placed at separate locations within the ARCA chamber during the decontamination run, while a single indicator strip was placed with the non-decontaminated control BI (Figure 1). Following the decontamination run, all indicator strips were visually inspected for a color change.

For decontamination, a VHP® 1000ED Biodecontamination System (STERIS Corporation) was connected to the ARCA chamber in which all airflow though the ARCA chamber had been shut off. The vaporous hydrogen peroxide supply was connected to one end of the ARCA chamber and the vaporous hydrogen peroxide return was connected to the opposite end of the ARCA chamber (Figure 1). The decontamination process consisted of dehumidification, conditioning, decontamination, and aeration phases, which were controlled by the hydrogen peroxide generator. Due to the size and complexity of the ARCA chamber, the established cycle parameters were based on the maximum amount of hydrogen peroxide (1,950 g) that could be utilized in a single run using the VHP® 1000ED. The specifics of each phase are provided in Table 1. During the decontamination run, the hydrogen peroxide concentration, relative humidity, and temperature were monitored in real-time using data loggers set to a data point capture frequency of 1 minute. The hydrogen peroxide was monitored using an ATI Series B12 two-wire gas transmitter connected to a 0-1000 ppm remote hydrogen peroxide sensor (Analytical Technology, Inc., Collegeville, PA). The sensor was suspended approximately 1 m from the middle of the ARCA main chamber ceiling. The temperature and relative humidity were monitored in real-time using a HOBO U12-006 data logger (Onset Computer Corporation, Bourne, MA) that was placed in the middle of the ARCA main chamber. A Yokogawa DX2010 (Yokogawa Electric Corporation, Tokyo) connected to an Omega HX93AC temperature/relative humidity probe (Omega Engineering Inc., Stamford, CT) was used to monitor temperature and relative humidity in real-time in the control chamber.
**Table 1**

Decontamination Parameters for the ARCA Chamber

<table>
<thead>
<tr>
<th>Phase</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehumidification</td>
<td>Duration: 10 min</td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 20 CFM</td>
</tr>
<tr>
<td></td>
<td>Relative Humidity: ≤6.9 mg/L</td>
</tr>
<tr>
<td>Conditioning</td>
<td>Duration: 20 min</td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 20 CFM</td>
</tr>
<tr>
<td></td>
<td>Injection Rate: 6.5 g/min</td>
</tr>
<tr>
<td>Decontamination</td>
<td>Duration: 5.5 hours</td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 20 CFM</td>
</tr>
<tr>
<td></td>
<td>Injection Rate: 5.0 g/min</td>
</tr>
<tr>
<td>Aeration</td>
<td>Duration: 30 min</td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 20 CFM</td>
</tr>
<tr>
<td>Additional Aeration</td>
<td>Duration: 4 hours</td>
</tr>
<tr>
<td></td>
<td>Flow Rate: 20 CFM</td>
</tr>
</tbody>
</table>

*CFM = cubic feet per minute*
Sample Processing and Analysis

Decontamination efficacy of *B. anthracis* spores was quantified by measuring the viable spores extracted from both decontaminated and control coupons as previously described (Rogers et al., 2005; Rogers et al., 2007). Briefly, after the decontamination run, test and control coupons were placed in a 50 ml conical tube containing 10 ml of sterile phosphate-buffered saline and agitated at 200 rpm on an orbital shaker for 15 minutes at room temperature. A 1.0 ml aliquot of each sample was removed, serially diluted 10-fold in sterile water, plated onto tryptic soy agar plates (Hardy Diagnostics, Santa Maria, CA) in triplicate, and incubated overnight at 37°C. Following an 18-24 hour incubation, plates were enumerated and CFU/ml determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data were expressed as the mean ± standard deviation (SD) of observed CFU. Decontamination efficacy was determined by calculating the log reduction in viable spores.

Following the decontamination run, BIs were placed into 15 ml conical tubes containing 10 ml of tryptic soy broth (Remel, Lexena, KS), gently agitated, and incubated at 55°-60°C. As the extraction process does not typically yield 100% recovery of spores, all material coupons were also placed in tryptic soy broth (TSB) and cultured to determine whether any viable spores remained on the coupons. All cultures were visually inspected for growth (cloudy culture) or no growth (clear culture) at 1 and 7 days post-decontamination as previously described (Rogers et al., 2005; Rogers et al., 2007). Streak plates were made of all positive cultures to verify the growing organism.

Results

Decontamination Run

During the conditioning phase, the hydrogen peroxide concentration peaked to approximately 460 ppm and decreased to a level of approximately 270 ppm during the decontamination phase. Figure 2 provides a representative hydrogen peroxide curve during an entire decontamination run. The relative humidity profile followed a curve similar to the hydrogen peroxide, peaking at approximately 95% and decreasing to a level of approximately 86% during the decontamination phase (Figure 3). The mean (±SD) temperature in the ARCA chamber during the decontamination run was 21.7° ± 0.3°C (Figure 3). In the control chamber, the mean (±SD) temperature and relative humidity were 24.2° ± 0.4°C and 46.2° ± 1.4%, respectively.

Following decontamination, all chemical indicator strips present in the ARCA chamber during the decontamination run changed color, indicating exposure to the vaporous hydrogen peroxide. The control chemical indicator strips did not change color. Upon visual inspection, no physical damage was observed for the hydrogen peroxide-exposed coupons.

Decontamination Efficacy

Exposure of test coupons contaminated with *B. anthracis* Ames, Vollum, or Sterne spores to vaporous hydrogen peroxide resulted in the inactivation of observable viable spores on all glass, Hypalon®, and stainless steel materials. For these three materials, the log reductions calculated from the number of viable spores recovered from each coupon inoculated with approximately
1 x 10⁸ CFU in 10 droplets ranged from 7.7 to 7.9, 8.2 to 8.5, and 7.6 to 7.8 for B. anthracis Ames, Vollum, or Sterne spores, respectively (Table 2). No viable organisms were detected from any of the blank samples.

All decontaminated material coupons cultured in TSB exhibited no growth at 7 days, while the control coupons were positive for growth at 1 day (Table 3). For all BI exposed to the vaporous hydrogen peroxide, no growth was observed as determined by the lack of visibly cloudy liquid cultures through 7 days post-exposure, while the unexposed control BI exhibited growth as determined by the presence of visibly cloudy cultures (Table 3). Streak plates of the positive cultures for the coupons and BI exhibited homogenous growth of B. anthracis and G. stearothermophilus, respectively. All blank coupons exhibited no growth through 7 days.

Discussion

Virulent B. anthracis spores have been decontaminated on a large scale to include buildings and facilities contaminated as a result of their intentional release in the mail in 2001 and at a textile mill that processed goat hair (Canter et al., 2005; Young et al., 1970). To our knowledge, the present study is the first laboratory-controlled, large-scale decontamination test using vaporous hydrogen peroxide against B. anthracis spores. The results of this study show that B. anthracis Ames, Vollum, or Sterne spores were inactivated on glass, Hypalon® rubber glove, and stainless steel surfaces after decontamination with vaporous hydrogen peroxide. The complete inactivation of these spore types is supported by the results in which no growth was observed from any of the material coupons during the quantitative counts of spores from the coupon extracts, and all liquid cultures of each decontaminated coupon were negative for growth. These results suggest that the decontamination parameters outlined in this study were effective in inactivating three strains of B. anthracis spores in a 520 ft³ (~14.7 m³) ARCA chamber using vaporous hydrogen peroxide.

The relationship between the response of virulent B. anthracis and surrogates deposited on various surfaces and treated with different types of decontaminants is not well-characterized. Although many studies regarding the decontamination of Bacillus spores have been published, most of the available data has been obtained from the use of B. anthracis surrogates such as B. atrophaeus, B. subtilis, B. anthracis Sterne, B. anthracis NNR1Δ1, and G. stearothermophilus (Rastogi et al., 2009; Rogers et al., 2008a; Spotts Whitney et al., 2003). With respect to fumigants, a couple of studies have directly compared the decontamination efficacy of B. anthracis and surrogate spores on porous and non-porous surfaces using vaporous hydrogen peroxide and formaldehyde (Rogers et al., 2005; Rogers et al., 2007). Comparative data have also been presented in studies evaluating the use of liquid decontaminants against B. anthracis and surrogate spores on non-porous materials (Majcher et al., 2008; Sagripanti et al., 2007). Although these studies provide useful comparative data on many surfaces with multiple types of decontaminants, the lack of data using virulent B. anthracis spores has increased the attention to filling data gaps by directly comparing biological agents and their surrogates. Therefore, in this study we chose to assess the inactivation of both virulent and avirulent B. anthracis spores in parallel as a means to have direct comparative efficacy data obtained from identical decontamination conditions in a large-scale laboratory test.
In this study, the material types chosen for the decontamination testing were representative of those comprising most of the surface area within the ARCA chamber, and the results show that *B. anthracis* spores on glass, Hypalon® rubber glove, and stainless steel materials can be inactivated. However, these materials are considered non-porous and may not be representative of additional materials that could be introduced into the ARCA chamber for research purposes. The decontamination efficacy of vaporous hydrogen peroxide has been shown to be affected by different factors, such as material porosity and spore density (Han et al., 2003; Rogers et al., 2005; Rastogi et al., 2009; Spiner & Hoffman, 1971; Young et al., 1970). For more complex and porous materials, the inoculated spores may penetrate and stratify within the test material and could interfere with the contact of the decontaminant with the spores, thereby potentially decreasing spore inactivation. Therefore, when decontaminating large-scale enclosures such as the ARCA chamber, laboratory, or room where re-entry is imminent, it is important to evaluate the inactivation of *B. anthracis* spores (and other biological agents) on material types that may be present in the enclosure to provide both best- and worst-case scenarios for challenging the decontamination process.

The generation of biological agent aerosols is one of the capabilities of the ARCA chamber that would be advantageous for conducting future decontamination research. An important factor that could potentially influence the decontamination efficacy of vaporous hydrogen peroxide or other fumigants is the method of spore deposition. In this study and as done previously (Rastogi et al., 2009; Rogers et al., 2005; Rogers et al., 2007), spores were delivered to the material surfaces as droplets from an aqueous suspension that were left undisturbed to dry. However, the 2001 intentional release of

### Table 2

Mean (± SD) Total Viable Colony-forming Units (CFU) and Mean Log Reduction of *B. anthracis* Ames, Vollum, and Sterne Spores Exposed to Vaporous Hydrogen Peroxide. Each coupon was inoculated with $1 \times 10^8$ CFU in 10 droplets.

<table>
<thead>
<tr>
<th>Spores/Test Material</th>
<th>Total Viable CFU Recovered (Mean ± SD)</th>
<th>Mean Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. anthracis</em> Ames</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>$4.65 \pm 1.24 \times 10^7$</td>
<td>≥7.7</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypalon® Rubber Glove</td>
<td>$7.47 \pm 0.78 \times 10^7$</td>
<td>≥7.9</td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Decontaminated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>$8.64 \pm 2.64 \times 10^7$</td>
<td>≥7.9</td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Decontaminated</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. anthracis</em> Vollum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>$2.74 \pm 1.18 \times 10^8$</td>
<td>≥8.4</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypalon® Rubber Glove</td>
<td>$3.49 \pm 2.90 \times 10^8$</td>
<td>≥8.5</td>
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<td>Unexposed Control</td>
<td>0</td>
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<tr>
<td>Decontaminated</td>
<td></td>
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<tr>
<td>Stainless Steel</td>
<td>$1.64 \pm 0.12 \times 10^8$</td>
<td>≥8.2</td>
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<td></td>
</tr>
<tr>
<td>Decontaminated</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. anthracis</em> Sterne</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>$4.32 \pm 2.38 \times 10^7$</td>
<td>≥7.6</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypalon® Rubber Glove</td>
<td>$6.00 \pm 1.17 \times 10^7$</td>
<td>≥7.8</td>
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<tr>
<td>Unexposed Control</td>
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<td></td>
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<tr>
<td>Decontaminated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stainless Steel</td>
<td>$5.37 \pm 0.35 \times 10^7$</td>
<td>≥7.7</td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Decontaminated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* “—” = Not applicable.
B. anthracis spores in the mail consisted of a fine, dry powder aggregate that formed an aerosol depositing over surfaces. It is possible that the spore preparation and delivery mechanism may have an effect on the decontamination efficacy of spores deposited as an aerosol compared to spores delivered as an aqueous suspension. Therefore, it is our intent to conduct future research in which spores aerosolized within the ARCA chamber will be allowed to settle onto the surface of material coupons and subsequently be decontaminated with a fumigant such as vaporous hydrogen peroxide. Such studies will enable a comparison that will address the question of whether differences exist in the inactivation of spores on surfaces that were deposited as an aerosol or dried from an aqueous suspension.

### Acknowledgments

We thank Robert Davenport for his technical assistance. This study was funded by Battelle’s Internal Research and Development Program.

### References


### Table 3

Qualitative Growth Assessments of Test Materials and Biological Indicators Exposed to Vaporous Hydrogen Peroxide

<table>
<thead>
<tr>
<th>Spores/Test Materiala</th>
<th>No. Tested (No. Positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td><strong>B. anthracis Ames</strong></td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td>Hypalon® Rubber Glove</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td><strong>B. anthracis Vollum</strong></td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td>Hypalon® Rubber Glove</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td>Stainless Steel</td>
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</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td><strong>B. anthracis Sterne</strong></td>
<td></td>
</tr>
<tr>
<td>Glass</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td>Hypalon® Rubber Glove</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td>Stainless Steel</td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>3(3)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>3(0)</td>
</tr>
<tr>
<td><strong>Biological Indicators</strong></td>
<td></td>
</tr>
<tr>
<td>Unexposed Control</td>
<td>1(1)</td>
</tr>
<tr>
<td>Decontaminated</td>
<td>16(0)</td>
</tr>
</tbody>
</table>

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


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¹Emory University Rollins School of Public Health, Atlanta, Georgia, and ²Vanderbilt University, Nashville, Tennessee

Abstract

Concern over the adequacy of biosafety training and incident-reporting practices within biological laboratories in the United States has risen in recent years due to the increase in research on infectious diseases and the concomitant rise in the number of biocontainment laboratories. Reports of laboratory-acquired infections and delays in reporting such incidents have also contributed to the concern. Consequently, biosafety training and incident-reporting practices are being given considerable attention by both the executive branch and Congress. We conducted a 51-question survey of biosafety professionals in June 2008 to capture information on methods used to train new laboratory workers within biosafety level 2 (BSL-2) laboratories, animal biosafety level 2 (ABSL-2) laboratories, biosafety level 3 (BSL-3) laboratories, and animal biosafety level 3 (ABSL-3) laboratories. The survey results suggest nearly all senior scientists, faculty, staff, and students working in these biocontainment laboratories are required to have biosafety training, and three-quarters of respondents indicated a biosafety or environmental health and safety professional provides explicit instructions on reporting incidents to each new lab worker. Only half of the respondents with BSL-2/ABSL-2 laboratories at their institution and 59% of respondents from institutions with BSL-3/ABSL-3 laboratories indicated custodial or maintenance workers are required to receive biosafety training at the BSL-2/ABSL-2 and BSL-3/ABSL-3 levels, respectively. Opportunities for targeted improvement such as providing training to non-traditional laboratory workers (e.g., custodians, maintenance workers) and posting laboratory incident-reporting protocols on institutional environmental health and safety websites may exist. Variations in biosafety training requirements, incident-reporting practices, and attitudes towards laboratory safety revealed through this survey of biosafety professionals also support the development of core competencies in biosafety practice that could lead to more uniform practices and robust safety cultures.

Background

In recent years, an increase in biodefense research, including research on emerging infectious diseases, has led to concomitant increases in the number of biocontainment laboratories in the United States and persons needed to staff them. In addition, the select agent legislation subsequent to the anthrax attacks provided more rigorous regulation of biosafety and biosecurity; the select agent regulations prior to this time had primarily regulated shipment and transfer of select agents (Dembek, 2007). Biosafety has become a highly visible issue nationally, especially for laboratories working on pathogens designated as select agents. Universities have received adverse publicity following reports of occupationally-acquired illnesses among laboratory workers and a significant delay in reporting those illnesses to public health authorities (Enserink, 2007; Kaiser, 2007; Lawler, 2005). Concerns that biosafety training is inadequate and reporting systems for laboratory incidents are insufficient have also increased. Both the executive and legislative branches of government have been considering actions to ensure biosafety and biosecurity in U.S. laboratories and have been considering how best to implement a reporting system for laboratory incidents (Executive Order, 2009; Graham et al., 2008; Rhodes, 2007; S.3127, 2008).

In an effort to assess laboratory incident-reporting practices, the Policy, Ethics and Law (PEL) Core of the Southeastern Regional Center of Excellence for Emerging Infections and Biodefense (SERCEB) convened a workshop on January 30, 2008. As one of 11 regional centers of excellence devoted to emerging infectious disease and biodefense research, the SERCEB consortium conducts research with infectious agents in laboratories meeting BSL-2 or BSL-3 criteria (National Institutes of Health, 2009). Biosafety professionals and researchers from seven SERCEB institutions, including University of North Carolina at Chapel Hill, Duke University, Vanderbilt University, Emory University, University of Florida, University of Alabama at Birmingham, and Wake Forest University, came together to review their laboratory incident-reporting structures and to collectively assess ways to improve reporting—not only to improve operations but also to enhance the support and management of biosafety in their institutions and the communities in which they operate.

To facilitate discussion at the workshop, a working definition of “incident” as it relates to laboratories working with infectious agents was provided to participants. This definition was “any occurrence that has the potential to lead to unintended exposure of the agent to hu-
mans, animals or the environment.” Workshop participants noted that this broad definition encompasses a range of events which often vary among institutions. Each institution can also have different reporting channels depending on the type of incident. Workshop participants did not reach consensus on what incident-reporting practices are considered standard or sufficient. Each representative described different methods used for encouraging laboratory workers to report incidents. Upon examination of the institutions’ incident-reporting protocols, the majority of participants agreed that a strong link exists between incident-reporting and biosafety training. If incidents can be utilized as lessons learned, they can serve to strengthen biosafety training and overall laboratory safety. Participants at the workshop believed the need to improve biosafety cultures across BSL-2 and BSL-3 laboratories regardless of the nature of the research is paramount. Participants agreed more data needed to be collected from biosafety professionals and laboratory workers in order to identify mechanisms that might improve biosafety training and incident-reporting practices, both within life science laboratories and across institutions.

This 51-question anonymous survey of biosafety professionals in the United States was developed to obtain information about biosafety training and incident-reporting practices within BSL-2 and BSL-3 laboratories. The goal was to identify aspects of the biosafety training and incident-reporting feedback loop that could inform industry-wide best practices to strengthen overall biosafety practices and reporting procedures.

Methods

This survey addressed biosafety training practices, biosafety compliance and oversight practices, incident-reporting and incident-reporting protocols, information sharing, and attitudes towards biosafety at respondents’ institutions. The survey allowed respondents to use their own institutions’ criteria for what constitutes an incident. Data were collected on BSL-2/ABSL-2 and BSL-3/ABSL-3 laboratories. No data were collected on BSL-4/ABSL-4 level laboratories; biosafety officials convened separately to address the biosafety training issues for these maximum containment facilities (Le Duc et al., 2008).

The survey was approved by the Emory University Institutional Review Board and piloted in April 2008 at the Emory Biosafety Leadership Institute in Atlanta, Georgia. The national survey was administered through the web-based survey tool SurveyMonkey.com (Finley, 2009) and was distributed by the American Biological Safety Association (ABSA) to its membership of approximately 1,700 individuals on June 16, 2008. The survey remained open for one month, until July 16, 2008. Eligible respondents were individuals at least 18 years of age and practicing biosafety professionals employed within the United States. The total number of ABSA members who meet these criteria is unknown.

Data were analyzed using SAS version 9.2 statistical software (SAS Institute, Cary, NC). Statistical analyses included χ² and Fisher’s Exact for cell values <5. A P-value < 0.05 was considered significant.

Results

Demographics

Approximately 19% (318/1700) of the total ABSA membership entered the online survey, and of these 318 individuals, 81% (258) met the survey’s eligibility requirements. Of these 258, 93% (240) provided responses to survey questions.

Fifty-two percent of the 240 respondents were from academia, 23% were employed by private companies or industry, 16% worked for U.S. Government agencies, and 10% worked for non-profit organizations. The majority of respondents (over 70%) categorized themselves as biosafety officers, environmental health and safety officers, or directors of environmental health and safety divisions. Approximately one-fourth provided other job titles, including occupational health and safety specialists, laboratory managers, and scientists.

Approximately 30% of respondents indicated they have at least one professional biosafety certification. Twelve percent of respondents indicated they are a Registered Biosafety Professional (RBP), and 11% indicated they are a Certified Biological Safety Professional (CBSP). Another 8% indicated they are both RBP- and CBSP-certified.

Regarding full-time equivalent (FTE) employees devoted to biosafety, most respondents indicated their institution has less than three FTEs devoted to biosafety (Table 1). Nearly all respondents (94%) from institutions with BSL-2/ABSL-2 or lower biocontainment laboratories reported having less than three FTEs devoted to biosafety. Of respondents from institutions with BSL-3/ABSL-3 laboratories, 64% indicated operating with less than three FTEs devoted to biosafety. For deidentification purposes, data were not collected on institution size or number of biocontainment laboratories; no correlations between number of FTEs devoted to biosafety and size of facilities were drawn.

Biosafety Training Practices

Training Requirements by Biosafety Level

Of 136 respondents with BSL-3/ABSL-3 laboratories at their institutions, nearly all indicated that senior scientists, faculty, laboratory staff, and students working in BSL-3/ABSL-3 laboratories are required to take biosafety training (Table 2). Among 202 respondents with BSL-2/ABSL-2 laboratories at their institutions, 85% and 91% indicated biosafety training at the BSL-2/ABSL-2 level is required of senior laboratory scientists/faculty and
laboratory staff/students, respectively. At both levels, a lower percentage of respondents indicated biosafety training requirements for other types of laboratory workers such as visiting scientists and custodial or maintenance workers.

Among 179 respondents who indicated their institutions require biosafety training at the BSL-2/ABSL-2 level, 64% (115/179) indicated new BSL-2/ABSL-2 laboratory workers are tested on their knowledge of biosafety techniques after having received training (Figure 1). At the BSL-3/ABSL-3 level, 88% (112/128) of respondents indicated new workers at the BSL-3/ABSL-3 level are tested on knowledge of biosafety techniques.

Didactic vs. Hands-on Training: Biohazardous Spills and Needlesticks

Of 188 respondents with BSL-2 laboratories at their institutions, 23% (44/188) indicated drills or activities simulating biohazardous spills are included in the required biosafety training of new laboratory workers entering BSL-2/ABSL-2 laboratories (Table 3). Spill training involving drills and simulations for BSL-2 laboratory workers was used more frequently if the institution also had a BSL-3/ABSL-3 laboratory.

Regarding hands-on training for response to needlestick incidents, 7% (13/184) of respondents from institutions with BSL-2/ABSL-2 laboratories indicated hands-on training is used to teach new laboratory workers how to respond to needlestick incidents at the BSL-2/ABSL-2 level. The percentage utilizing hands-on training for needlesticks was higher among those respondents whose institutions also have BSL-3/ABSL-3 laboratories.

For training new laboratory workers entering BSL-3/ABSL-3 laboratories, 64% (82/128) and 25% (30/121) of respondents indicated drills or simulations are included in the required biosafety training for biohazardous spills and needlestick incidents, respectively.

For workers in both BSL-2/ABSL-2 and BSL-3/ABSL-3 laboratories, didactic training is the most common method for needlestick training, with 45% (83/184) and 36% (44/121) of respondents indicating didactic training is the only method used to train new laboratory workers about needlestick incidents at the BSL-2/ABSL-2 and BSL-3/ABSL-3 levels, respectively. Respondents indicated that demonstrations by video or e-module are used more frequently at the BSL-2/ABSL-2 level, whereas demonstrations by the biosafety officer are used more often at the BSL-3/ABSL-3 level.

Table 1

Number of full-time equivalent (FTE) employees devoted to biosafety at institutions with and without BSL-3/ABSL-3 laboratories.

<table>
<thead>
<tr>
<th>Number of FTEs devoted to biosafety</th>
<th>Institutions with BSL-2/ABSL-2 laboratories; no BSL-3/ABSL-3 laboratories (n=69)</th>
<th>Institutions with both BSL-2/ABSL-2 and BSL-3/ABSL-3 laboratories (n=151)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>36 (52%)</td>
<td>21 (14%)</td>
</tr>
<tr>
<td>1 - &lt;3</td>
<td>29 (42%)</td>
<td>75 (50%)</td>
</tr>
<tr>
<td>≥3</td>
<td>4 (6%)</td>
<td>55 (36%)</td>
</tr>
</tbody>
</table>

Table 2

Types of laboratory workers required to take biosafety training by biosafety level of laboratory.

<table>
<thead>
<tr>
<th></th>
<th>BSL-2/ABSL-2 laboratories (n=202)</th>
<th>BSL-3/ABSL-3 laboratories (n=136)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senior laboratory scientists and/or faculty</td>
<td>85%</td>
<td>99%</td>
</tr>
<tr>
<td>Laboratory staff and/or students</td>
<td>91%</td>
<td>99%</td>
</tr>
<tr>
<td>Visiting: scientists, students, and/or faculty</td>
<td>72%</td>
<td>86%</td>
</tr>
<tr>
<td>Custodial or maintenance workers</td>
<td>50%</td>
<td>59%</td>
</tr>
<tr>
<td>Biosafety training is not required at this level</td>
<td>8%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Annual Laboratory Inspections and Renewal of Biosafety Training Requirements

Ninety-three percent (119/128) of respondents with BSL-3/ABSL-3 laboratories at their institutions indicated that these laboratories receive an annual inspection by a biosafety officer. Of the 119 respondents, 87% (104/119) indicated their institutions have one or more FTE employees dedicated to biosafety, whereas 44% (4/9) of the respondents who indicated the level-3 laboratories at their institutions do not receive an annual inspection by a biosafety officer have one or more FTE employees dedicated to biosafety. This correlation between number of FTEs devoted to biosafety and a yearly BSL-3/ABSL-3 laboratory inspection is significant (P=0.0048).

At the BSL-2/ABSL-2 level, no correlation between number of FTEs devoted to biosafety and yearly laboratory inspection was found. Seventy-three percent (141/193) of respondents with BSL-2/ABSL-2 laboratories at their institutions indicated each BSL-2/ABSL-2 laboratory undergoes an annual inspection by a biosafety officer. Of these 141 respondents, 73% (103/141) have one or more FTE employees devoted to biosafety. Sixty-nine percent (36/52) of respondents who indicated their level-2 laboratories do not get inspected annually by a biosafety officer indicated their institutions also have one or more FTE employees devoted to biosafety.

Forty-seven percent of respondents (93/197) indicated their institutions require all laboratory workers in the life sciences to renew their biosafety training requirements annually. Thirty-one percent of respondents (62/197) indicated selected laboratory workers are required to renew their biosafety training annually, with the major-
ity of these workers identified as those involved in blood-borne pathogen research, select agent research, or working within BSL-3/ABSL-3 laboratories. Twenty-one percent of respondents (42/197) indicated annual renewal of biosafety training is not a requirement at their institutions.

Of the 93 respondents who indicated that their institutions require all laboratory workers to renew biosafety training annually, 87% (81) indicated having a positive perception of overall safety within the laboratories they work with or oversee. Perception of safety was determined by degree of agreement or disagreement with the statement, “I feel like the laboratories I oversee or work with are safe places to work.” Of the 62 respondents who indicated that selected laboratory workers are required to renew their biosafety training annually, 69% (43) indicated having a positive perception of overall safety; of the 42 respondents who indicated that annual biosafety training renewal is not required of any laboratory workers, 62% (26) indicated having a positive perception of overall laboratory safety in the laboratories they work with or oversee.

**Biosafety Compliance and Annual Individual Performance Evaluation**

Of 174 individuals who indicated they oversee a BSL-2 laboratory or higher:
- 35% (61) indicated that some or all faculty or senior researchers are evaluated annually on their compliance with safety protocols
- 50% (87) indicated that at some or all of the staff within the laboratories that they work with or oversee are evaluated annually on their compliance with safety protocols
- 21% (36) indicated biosafety compliance is part of the annual performance evaluation for all students working in laboratories which they work with or oversee
- 34% (60) indicated that compliance with biosafety measures is not part of any laboratory workers’ annual individual performance evaluations
- 13% (23) were not sure whether or not compliance with safety measures is part of the annual individual performance evaluation

**Incident-reporting Protocols**

**Developing Incident-reporting Protocols**

Approximately 69% of respondents (132/190) indicated their biosafety office and/or environmental health and safety office creates and maintains their institutions’ formal written protocols for how to report incidents across all laboratories. One-quarter of respondents (47/190) indicated creating and maintaining incident-reporting protocols was the responsibility of another administrative division (e.g., occupational health and safety), and about 6% (11/190) revealed each research division or individual laboratory is responsible for creating and maintaining its own incident-reporting protocols.

Seventy-seven percent of respondents (156/201) indicated that a biosafety/environmental health and safety professional at their institutions provides explicit instructions on reporting incidents to each new laboratory worker.
Communicating Incident-reporting Protocols and Lessons Learned

Approximately three-quarters of respondents indicated protocols for reporting biohazardous spills and needlestick incidents are posted directly on their biosafety/environmental health and safety websites or accessible via a clear link that is provided on the safety site (Table 4). Approximately 44% of respondents indicated they post or provide links for reporting protocols on theft or vandalism to a life science laboratory.

Sharing Lessons Learned

Forty-four percent of respondents (90/207) indicated that within the last 12 months, their institutions have created and disseminated at least one newsletter, e-newsletter, or flyer that addressed biosafety issues. Seventy percent (63/90) of these respondents indicated their newsletter is used to share information about laboratory incidents or lessons-learned from incidents. Sixty of these 63 respondents also responded to the question on perception of safety. Ninety percent (54/60) of these individuals indicated a positive perception of safety within their laboratories versus 69% (11/16) of those whose institutions do not share lessons learned in their newsletter (P = 0.047).

Relationship Between Life Science Laboratories and Medical Support Staff

Nearly half of survey respondents (93/196) characterize the relationship between the life science laboratories and medical support staff at their institutions to be “close” or “very close.” The remaining 53% (103/196) of respondents gave ratings of “somewhat close,” “not close,” or “distant.”

Of the 93 respondents who indicated a “close” or “very close” relationship between the life science laboratories and the medical support staff, 92% (86) gave favorable ratings regarding their perception of overall safety within the laboratories they work with or oversee. In contrast, 64% (66/103) of the respondents who rated their relationship with medical support staff as “somewhat close,” “not close,” or “distant” gave favorable ratings on their perception of overall safety within the laboratories they work with or oversee (P < 0.0001).

**Table 4**

Posting formal incident reporting protocols on biosafety/environmental health and safety websites.

<table>
<thead>
<tr>
<th>Incident Type</th>
<th>Yes</th>
<th>No, but a clear link to the guidance is provided</th>
<th>No</th>
<th>Not sure/No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biohazardous spills</td>
<td>70% (138)</td>
<td>5% (9)</td>
<td>19% (38)</td>
<td>6% (11)</td>
</tr>
<tr>
<td>Any needlesticks</td>
<td>70% (137)</td>
<td>8% (15)</td>
<td>18% (35)</td>
<td>5% (9)</td>
</tr>
<tr>
<td>Contaminated needlesticks</td>
<td>66% (129)</td>
<td>7% (14)</td>
<td>18% (36)</td>
<td>9% (17)</td>
</tr>
<tr>
<td>Laboratory-acquired infections</td>
<td>59% (115)</td>
<td>8% (15)</td>
<td>24% (48)</td>
<td>9% (18)</td>
</tr>
<tr>
<td>Animal bites or scratches</td>
<td>54% (106)</td>
<td>8% (16)</td>
<td>27% (53)</td>
<td>11% (21)</td>
</tr>
<tr>
<td>Equipment failure that results in potential loss of containment</td>
<td>49% (96)</td>
<td>6% (12)</td>
<td>35% (68)</td>
<td>10% (20)</td>
</tr>
<tr>
<td>Break or tear in Personal Protective Equipment (PPE)</td>
<td>42% (83)</td>
<td>5% (10)</td>
<td>39% (76)</td>
<td>14% (27)</td>
</tr>
<tr>
<td>Unauthorized entry to a life sciences laboratory</td>
<td>38% (75)</td>
<td>9% (18)</td>
<td>38% (75)</td>
<td>14% (28)</td>
</tr>
<tr>
<td>Theft or vandalism within a life sciences laboratory</td>
<td>35% (68)</td>
<td>9% (18)</td>
<td>39% (77)</td>
<td>17% (33)</td>
</tr>
</tbody>
</table>
Discussion

Because this survey was distributed only to members of the American Biological Safety Association and some respondents’ surveys were not entirely complete, the results may not be representative of the larger community of biosafety professionals or biocontainment laboratories. This survey also does not present perspectives from other cohorts of individuals involved in the oversight of laboratory research, such as laboratory workers.

Additionally, the survey was limited to biosafety professionals working within the United States, so no international perspectives are represented. Nevertheless, survey findings are sufficiently robust to demonstrate both substantial attention to biosafety and opportunities to strengthen specific aspects of biosafety training and incident-reporting practices.

The results from this survey suggest that nearly all senior scientists, faculty, staff, and students working in BSL-2/ABSL-2 and BSL-3/ABSL-3 laboratories are required to have biosafety training. The vast majority of BSL-3/ABSL-3 laboratories get inspected at least once per year by a biosafety officer, and nearly three-quarters of respondents indicated their biosafety or environmental health and safety website posts incident-reporting protocols on biohazardous spills and needlesticks. Over three-quarters of respondents also indicated a biosafety or environmental health and safety professional provides explicit instructions on reporting incidents to each new laboratory worker. At the same time, the results identified biosafety training and incident-reporting practices could be improved in many laboratories through targeted measures at the institutional level.

Considering Workers’ Roles, Training Priorities, and Core Biosafety Competencies

Determining which individuals within a research institution should receive biosafety training and instruction on incident-reporting is a necessary component of any biosafety program. As biological research programs increase in number and size, new and larger cohorts of people, including maintenance workers, custodians, and visiting scientists, require access to laboratories. However, due to their varied roles within a laboratory, each of these groups is likely to require tailored biosafety training. Determining adequate training for these individuals is a challenge for biosafety professionals. Results from this survey indicate many institutions do not require custodial or maintenance workers to have biosafety training. Over one-quarter of institutions with BSL-2/ABSL-2 laboratories also do not require visiting scientists to have biosafety training. While this survey did not inquire about biosafety training for local police, fire, or EMS responders, increasing numbers of laboratories are beginning to provide training for these individuals as well (Kaufman et al., 2009).

Adequate training on biosafety concepts also involves determining which training methodologies result in the greatest absorption and retention of concepts taught. While certain activities such as biohazardous spill training may benefit the worker most when taught via hands-on simulations, other training components may be readily retained when described verbally by a biosafety professional or other appropriate instructor. While the 5th edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* guidance encourages evaluation of staff proficiency regarding safe practices in BSL-2 through BSL-4 laboratories, it neither describes effective ways to train on specific concepts, nor suggests ways to assess those competencies (U.S. Department of Health and Human Services, CDC & NIH, 2007). To date, these decisions have been left up to individual laboratories. Such autonomy may have been adequate in past decades, but with growth in the number of laboratories and laboratory personnel, more standardization around core competencies at various biosafety levels would be useful.

The data from this survey also suggest that biosafety professionals believe the laboratories they work with or oversee are safer places to work when laboratory workers are required to renew their biosafety training requirements annually. Periodic renewal of biosafety training may be important in reinforcing institutional safety expectations and providing an opportunity to review new safety measures (Isouard, 1988). While proactive outreach by laboratory workers to biosafety professionals is ideal, many laboratory workers may not seek clarification on safety-related concerns from institutional biosafety or environmental health and safety offices. Establishing periodic renewal periods can enable biosafety professionals to reserve time with busy laboratory workers to clarify concepts and answer safety-related questions.

Encouraging Incident-reporting at the Institutional Level Through Translation into Lessons Learned

Studies conducted throughout the 20th century indicated that surveillance systems for laboratory-acquired infections and incidents leading to possible exposures are not robust (Pike, 1976; Pike, 1979; Sejvar et al., 2005; Sewell, 1995). However, incident reports begin at the institutional level, and the lack of reporting at various levels both within the institution and external to the institution can be due to a variety of reasons separate from the existence and availability of standardized reporting protocols. Laboratory workers may be reluctant to report due to embarrassment, fear of retribution, or the belief that an incident was not worthy of reporting. Other studies have also cited inadequate feedback to the persons reporting incidents as a major deterrent to reporting incidents in the workplace (Evans et al., 2006; Handler et al., 2007). While there are many ways to pro-
vide feedback, turning incidents into lessons learned is one method. This survey inquired about whether or not lessons learned from incident reports are ever shared in biosafety newsletters or flyers. A higher percentage of survey respondents indicated a positive perception of laboratory safety if they used their newsletters to share lessons learned from incidents versus those who indicated that their newsletters were not used to share lessons learned. Reporting incidents is a behavior, and determining ways to turn reported incidents into lessons learned may be one way to decrease fear or negative stigma associated with reporting.

**Promoting Buy-in and Support of Biosafety Practices from Laboratory Directors and Principal Investigators**

The commitment of senior leadership to workplace safety practices is well regarded as an important factor in cultivating and promoting workplace safety (Andriessen, 1978; DeJoy, 2005; Simard & Marchard, 1995; Thompson et al., 1998). If senior leadership is not committed to safety or if senior leadership is not held to the same safety standards as its staff, workers may have less motivation to adhere to more stringent safety requirements. While 50% of survey respondents who oversee a BSL-2/ABSL-2 or higher laboratory indicated that some or all staff within the laboratories they work with or oversee are evaluated annually on their compliance with safety protocols, only 35% (61/174) indicated that some or all faculty or senior researchers are evaluated annually on their compliance with safety measures. Moreover, survey data suggest that slightly more laboratory staff and students are required to have biosafety training at the BSL-2/ABSL-2 level than senior laboratory scientists or faculty at the same level.

Keeping job requirements in mind is important when considering who should be required to take biosafety training, when they should renew, and whether or not it should be an element built into their performance evaluations. With some rarely engaging in laboratory activities, many senior scientists and principal investigators (PIs) commonly delegate research duties to their laboratory staff. Moreover, evaluations of research PIs may be done by department chairpersons who may or may not have the ability to evaluate adherence to biosafety practices. Regardless, it remains important to acknowledge senior scientists’ roles both within their laboratories and within their institutions broadly. Within their laboratories, despite their presence or absence from the bench, many senior scientists are considered the responsible representative for their laboratories. They are held accountable for ensuring that their staff is properly trained, and they are responsible for preventing and responding to incidents within their laboratories. Within their institutions, they are the key link between the laboratory and institutional leadership and play a key role in conveying the importance of a well-supported laboratory safety program.

**Examining and Improving Laboratory Relationships with Medical Support Staff**

The *BMBL* puts a significant emphasis on the role of medical support services in the promotion of a safe working environment within laboratories. Section VII of the *BMBL* focuses on occupational health, citing that optimal worker protection depends on the effective, ongoing collaboration among principal investigators, laboratory directors, safety specialists, and healthcare providers (U.S. Department of Health and Human Services, CDC & NIH, 2007). Approximately half of the biosafety professionals who responded to this survey described the working relationship between life science laboratories and health care providers (i.e., medical support service) at their institution as “somewhat close,” “not close,” or “distant.” Considering that this survey asked only one question about this relationship, this would be an interesting topic that would benefit from further inquiry and to determine measures that could strengthen this critical relationship.

Although this survey did not address it, a related laboratory issue of increasing concern is the interpersonal or emotional well-being of laboratory workers. Guidance on how to report and manage mental, emotional, or behavioral concerns in laboratory colleagues is becoming an important topic for biosafety officers, especially at institutions with biocontainment facilities. Further research and surveys are needed to explore the methods different institutions use to enhance personnel reliability.

**Conclusion**

This survey represents a cursory exploration into the biosafety training and incident-reporting practices within biological laboratories in the United States. Every institution that conducts biological research has different needs for biosafety, but certain practices such as posting incident-reporting protocols in accessible places such as biosafety websites and turning laboratory incidents into shared lessons learned are likely to improve biosafety regardless of the biosafety level of the laboratory. Engaging laboratory leadership in biosafety training activities and providing job-specific training to all persons entering biocontainment laboratories are also important to promoting a collective responsibility towards safety. Given the variety of individuals working in biological laboratories now, perhaps the time is right to develop industry-wide biosafety competencies that offer more detailed and uniform guidance about what each laboratory worker should be expected to know and demonstrate. As government and institutional leaders look for ways to make biological laboratories safer, these concepts are ones that deserve more attention, promotion, and research.
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Consequences of Failure to Apply International Standards for Laboratory Biosafety and Biosecurity: The 2007 Foot-and-Mouth Disease Outbreak in the UK

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Abstract

International standards have been elaborated for laboratory biosafety and biosecurity in relation to work on both human and animal pathogens. Comparing the findings from the two official reports (HSE, 2007; Spratt, 2007) regarding the 2007 Foot-and-Mouth Disease outbreak in the United Kingdom (UK) with these international standards highlights several areas of deficiency in biosafety and biosecurity standards in laboratory facilities and in national guidance and procedures, suggesting that had the international standards been fully applied, the outbreak would not have occurred.

Introduction

In August and September 2007, eight farms in Surrey and Berkshire (southern England) were affected by an outbreak of Foot-and-Mouth Disease (FMD). Two official investigations that rapidly took place established a very high likelihood that the outbreak was caused by inadvertent release of FMD virus from the nearby Pirbright laboratory site, where three groups were working with similar strains of the virus.

The World Health Organization (WHO) and the Office International des Epizooties/World Organization for Animal Health (OIE) publish standards for laboratory biosafety and biosecurity. This article examines whether the outbreak would have occurred had these standards been appropriately applied. International standards are important in this area because of the transboundary nature of infectious disease and related control efforts. One of the groups at the Pirbright site—the Institute for Animal Health—is an OIE World Reference Laboratory for FMD; therefore, consideration of OIE standards is particularly relevant to this case.

Background

Foot-and-Mouth Disease

FMD is highly contagious among cloven-hoofed animals, infecting up to 100% of livestock exposed to it. It is potentially massively economically damaging as measures to contain its spread affect both domestic and export markets. The UK National Audit Office estimates that a 2001 FMD outbreak cost the UK £8 billion (2007, p. 1). FMD is an OIE “listed disease”—“a transmissible disease with high potential for international spread”—for which it advises particular control measures. Due to its virulence and economic significance, OIE classifies FMD as a Category or Group 4 animal pathogen, which should be worked on only within maximum containment/ biosafety level 4 laboratory conditions. “OIE guidelines for the containment level for Group 4 pathogens are generally equal to the USDA’s biosafety level 3Ag guidelines” (OIE, 2008, Chapter I.1.02).

Outbreak and Response

On 3 August 2007, FMD was confirmed in cattle on a farm in Surrey. Given the stage of infection and the incubation period of the virus (14 days), the most likely period of exposure was identified as 14-26 July (HSE, 2007, para. 28). The UK government acted rapidly to contain the outbreak with reasonable success (although it did spread to several sites nearby) and fulfilled its international reporting requirements (OIE, 2007, Chapter 1.2.2).

The FMD strain was identified as OIBFS67, responsible for an FMD epidemic in the UK in 1967. The strain is no longer naturally occurring but was worked on at the nearby Pirbright laboratory site during the infection window. Investigators concentrated on examining this site as the likely source of the outbreak.

Biosafety and Biosecurity

As used internationally in relation to laboratory facilities, biosafety and biosecurity are distinct terms. Biosafety aims to prevent accidental/unintentional exposure to or release of pathogens within or from the laboratory environment. Biosecurity aims to prevent deliberate release or theft of pathogens from laboratory facilities.

Sources

The reports of the two official investigations into the cause of the outbreak—the Health and Safety Executive’s Final Report on Potential Breaches of Biosecurity at the Pirbright Site and the Independent (Spratt) Review of the Safety of UK Facilities Handling Foot-and-Mouth Disease Virus—are used as sources of information about conditions at the Pirbright site that may have contributed to the outbreak. Two further reviews (the Callaghan Review and the Anderson Review) were also consulted, but do not directly relate to conditions at the site.
The international standards/guidelines consulted include OIE’s Terrestrial Animal Health Code (TAHC) and the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual); and the WHO’s Laboratory Biosafety Manual and Biorisk Management: Laboratory Biosecurity Guidance. The 2007 edition of the Terrestrial Code and 5th edition of the Terrestrial Manual are used in this article because they were the versions available at the time of the outbreak. 2008 versions of both have since been published, with some changes to both structure and content.

Deficiencies in Biosafety and Biosecurity

Comparing conditions at the Pirbright site with the international standards is used to draw insights in three main areas:

1. Errors in biosafety and biosecurity at the site that may have led to the release, and points at which the standards were not met
2. Possible deficiencies in national governance, particularly points at which national rules, policies, and procedures did not meet international standards (These have been reviewed since the outbreak and changes are in progress.)
3. Lessons in terms of new issues/areas for inclusion in the international standards

Main Findings of the Official Investigations

The two main investigations found that there was a very high likelihood that the Pirbright site was the source of the outbreak: “There is very little doubt that the FMD outbreak was caused by Foot-and-Mouth Disease virus (FMDV) from one of the two facilities at Pirbright” (Spratt, 2007, p. 5). Three groups at the site—the Institute of Animal Health (IAH), Merial Animal Health Ltd., and Stabiltech—were working on closely related strains of the virus.

The most likely route of transmission from the site to livestock identified in the reports was the passage of vehicles associated with construction work at the site, through infected soil or water around the area of an exposed and broken effluent pipe, carrying infected material off-site and past nearby farms. The reports were confident that the release was inadvertent because it did not follow patterns of a deliberate release; however, both reports noted deficiencies that could have provided determined individuals an opportunity to access the virus (Spratt, 2007, para. 11, 28; HSE, 2007, para. 13, 46, 200). They also identified a range of biosafety deficiencies at the site. In several instances these did not breach national rules and procedures, despite clearly falling below international standards.

Comparison of Conditions at Pirbright with International Standards for Biosafety and Biosecurity

Use of a decontamination process that did not guarantee virus deactivation and subsequent discharge of effluent that potentially contained live virus

International standards relating to biosafety level 4 containment require complete virus deactivation prior to release of effluent. The Terrestrial Code summarizes laboratory requirements for different containment groups. It specifies that liquid effluent must be sterilized and monitored (Chapter 1.4.5). By definition, sterilization requires that all living organisms are killed. Chapter 1.1.6 of the Terrestrial Manual lists as an “essential requirement for all work” that “no infectious material should be discarded down laboratory sinks or any other drain.”

Likewise, the WHO Laboratory Biosafety Manual states that “the overriding principle is that all infectious material should be decontaminated, autoclaved or incinerated within the laboratory” (p. 17).

The Pirbright laboratories used a system of chemical decontamination (HSE, 2007, para. 139-152). The system falls short of international guidance, with the HSE Report raising “concerns as to whether a system of chemical treatments could ever be considered to sterilise liquid waste” (para. 137) and stating that the chemical system “does not achieve complete inactivation” (para. 8).

Damaged and leaking drainage system and poor maintenance and inspection regimes for effluent drains

It appears to have been recognized that live virus might enter the site’s effluent drainage system. The Department for Environment, Farming, and Rural Affairs (Defra) considered that the drainage system was part of Pirbright’s Category 4 containment zone. At the time of the outbreak, Defra was the licensor, regulator, and inspector for the laboratories. The HSE reported:

Weaknesses were identified in the containment standard of the effluent drains across the Pirbright site. These included displaced joints, cracks, debris-build up and tree root ingress...recordkeeping, maintenance and inspection regimes were considered inadequate” (paragraph 153) and that the drainage system was not “demonstrably leakproof”; “airtight or negatively pressured”; “proofed against ingress or egress of insects”; or “isolated from flooding. (Annex, p. 57)

The international standards do not expect live virus to be present in the drainage system and do not address the issue of their condition. The standards do state, for example, that “production facilities have to be designed in such a way that contamination of the external environment is prevented” (TAHC, Chapter 1.1.7). Thus, it is clear that the failure of containment in the site’s drainage system falls short of international standards.
Poor recordkeeping of human and vehicle movements into and around site and no proper control of access to site or into its restricted areas

Controlled access to restricted areas is important for biosafety and biosecurity reasons. Only appropriately trained and authorized personnel should be able to access restricted areas, and access should be properly documented so that all materials and movements can be traced if problems subsequently arise. Recommendations on these issues can be found in the international guidance. The Laboratory Biosecurity Guidance’s program of accountability involves “identification and selection of personnel with access” to dangerous pathogens (WHO, 2008, p. 8); the Laboratory Biosafety Manual advises that “only authorized persons should be allowed to enter the laboratory working areas” (p. 10); and limited personnel access is part of the TAHC’s level 4 requirements (Chapter 1.4.5.5).

The HSE Report found that “not all human and vehicle movements via the IAH gatehouse to the site were recorded”; “evidence of poor monitoring and control of access to restricted areas”; and “vehicles involved in construction” were likely to have unrestricted access to the site (para. 12, 13, 15). Additionally, the report noted:

Access to the main restricted area is through a single self-closing door, which is protected by digital lock entry. The digital code has not been changed in years. No log is kept on a day-to-day basis of who has entered the high containment facility. (para. 200)

Poor siting of HEPA filters, meaning that tests could not be conducted to standard

HEPA (high energy particulate air) filters play an important role in containment, ensuring that only air from which all pathogens have been removed leaves the laboratory. At level 4 the Laboratory Biosafety Manual instructs that “both supply and exhaust air must be HEPA-filtered” (p. 26). Specific requirements depend on whether positive pressure suits or biosafety cabinets are used for primary containment. The Pirbright laboratories used cabinets. For these, supply air must be HEPA-filtered and “exhaust air...must pass through two HEPA filters prior to release outdoors” (p. 26). The filters “need to be tested and certified annually” (p. 27).

The HSE Report indicates that the main laboratory may not have met these standards. “We have concerns about the filter arrangements throughout the main laboratory...it does not allow both filters to be tested independently; therefore, it cannot be guaranteed that both filters are working at any one time” (para. 108).

Potential for positive pressurization of laboratories

Biosafety level 4 requires maintenance of negative air pressure so that air will be drawn into the laboratory from areas of higher pressure—rather than travelling outwards—contributing to containment by preventing the release of unfiltered air. The Laboratory Biosafety Manual includes advice that “the building ventilation system must be so constructed that air from the containment laboratory...is not recirculated to other areas of the building” (p. 21); “laboratory doors should be kept closed” (p. 10); and in level 4 laboratories using biosafety cabinets, entry should be through two doors to maintain separation from the rest of the facility (p. 25).

Two main issues at the Pirbright laboratories had potential to cause positive pressurization. First, laboratory doors were left open. “When lab doors were left open (as we observed when laboratory work was in progress), the pressure in the labs would effectively become the same as in the corridor” (HSE, 2007, para. 102). Second, in some of the laboratory spaces, air leaked between rooms. “Our investigations revealed that some individual laboratories could become positively pressurized when the doors were closed and there was leakage of air between laboratories through unsealed pipe ducting” (HSE, 2007, para. 102). The HSE Report also noted problems of air leakage due to the condition of the building. “The fabric of the building was poor, with visible cracks in the walls and ceilings, and leak points around some windows” (para. 104).

Lack of vehicle decontamination

Given that the likely route of transmission involved transfer on vehicle tires, it is significant that “there was no evidence of wheel washes or basic cleaning of vehicles” (HSE, 2007, para. 222). The Terrestrial Code Appendix 3.6.1.1 (OIE, 2007) recommends “washing and disinfecting the outside of vehicles”.

Comparison of National Rules and Procedures with International Standards for Biosafety and Biosecurity

Unclear distinction between laboratory biosafety and biosecurity

Defra and the HSE Report were unclear on the distinction between laboratory biosafety and biosecurity. This may well result in laboratories’ uncertainty about what standards they ought to apply. The terms are clearly defined and distinguished in the international standards. While they can be mutually supportive, they have different aims, and the distinction is important. The HSE Report states:

There is no accepted definition of “biosecurity.” For the purposes of this report, the term will cover the implementation of a combination of containment measures and working practices, supplemented by management controls, to prevent inadvertent exposure of susceptible species to biological agents. (para. 37)

The focus on unintentional exposure or accidental release, according to the international standards, falls under the definition of biosafety not biosecurity:
Laboratory biosafety describes the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

Laboratory biosecurity describes the protection, control and accountability for valuable biological materials...within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release.” (WHO, 2006, Biorisk management: Laboratory biosecurity guidance, para. iv)

For example, the HSE states (para, 5):

We looked at biosecurity controls in four areas where we judged it possible for the virus to escape containment arrangements at Pirbright, namely solid waste disposal, airborne routes through the fabric of site buildings or faults in filtration systems, liquid waste disposal, and human movements.

In this case, they were looking at routes of accidental release and were considering biosafety controls. The Spratt Review distinguished the terms in the same way as the international standards.

Confusion over requirements for work with animal pathogens and standards of containment

There appears to have been confusion about what standards apply to laboratory work on animal pathogens and whether these differ from those on human pathogens. This is outlined in the following paragraphs from the HSE Report:

45. The guidance states that the containment requirements are based on those published by the Advisory Committee on Dangerous Pathogens (ACDP) as being suitable for work with ACDP Category 4 human pathogens.... This is then qualified by the following statement: “However, it should be noted that the Defra categorisation of pathogens and conditions of containment differ in points of detail from those published by ACDP. The reason for this is that ACDP is concerned with protection of workers in the workplace, whereas Defra is concerned with protection of livestock and the environment. Laboratories must meet Defra containment requirements to be considered for licensing under the Specified Animal Pathogens Order 1998 (SAPO). In addition the relevant ACDP requirements apply.”

46. It is our opinion that this statement can cause confusion as to the standards required. And: “Although the guidance states that the containment is based on ACDP Level 4, the practical reality is quite different. This may be because the organisms being used are exclusively animal pathogens, and controlling worker exposure is not considered a priority. ...At IAH Pirbright, the culture is quite different from that observed in ACDP Containment Level 3 or 4 laboratories.... The practical application of physical and procedural approaches to containment varied considerably between the Defra (SAPO) and ACDP standards.” (para. 46)

According to the international standards, both animal and human exposure should be considered when categorizing pathogens for laboratory work, in case either requires additional measures to be taken. As FMD does not affect humans (although they can carry the virus), “the principal purpose of containment is to prevent the escape of the pathogen from the laboratory into the national animal population” (TAHC, Chapter 1.4.5.5). This may appear to leave some uncertainty as to whether standards for containment of human and animal pathogens should generally be the same. At biosafety level 4 the international requirements are equally stringent. The Laboratory Biosafety Manual includes in Risk Group 4 any pathogen “that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly” (p. 1). Its requirements at each biosafety level make no distinction between work on human or animal pathogens.

Under the international standards, all work on FMD must take place under maximum containment conditions, regardless of whether the primary aim is prevention of human exposure or prevention of release into the animal population—with maximum containment conditions no less stringent for animal pathogens than for human ones.

Rules unable to identify some key points as biosafety breaches

Some of the areas in which the Pirbright facilities fell short of international standards do not appear to have been identifiable as biosafety breaches under the national rules. For example, in regard to inactivation processes used at Pirbright, the HSE Report found these “fully in compliance with their SAPO [Specified Animal Pathogen Order] requirements” with the presence of live virus in effluent “in accordance with Defra’s requirements” and the conclusion that “this act of discharge was permitted by Defra, hence...there was no breach of biosecurity at this juncture” (para. 16, 8, 9).

Inspection and licensing procedures allowed site to continue operating despite deficiencies

Defra was the licensor for the site under the Specified Animal Pathogens Order 1998 (SAPO). Licenses are renewed on a 5-year basis, thorough inspections take place before they are issued, and there are subsequent annual inspections (HSE, 2007, para. 40, 41).

The Laboratory Biosafety Manual expects regular inspection and licensing activities to involve “systematic examination of all safety features and processes within the laboratory” (p. 36), including procedures for decon-
tamination of waste, and that “certification of the laboratory should not be completed...until deficiencies have been adequately addressed” (p. 37). Similar points are made in the Terrestrial Code:

A laboratory should be allowed to possess and handle animal pathogens in group 3 or 4 only if it can satisfy the relevant authority that it can provide containment facilities appropriate to the group. (Article 1.4.5.6)

Concern over standards used for licensing is raised given that Defra had licensed both Merial and IAH work at the site (Stabilitech was operating under the IAH license), particularly since the reports pointed to many of the problems at the site not being new but related to the age and condition of the buildings and lack of maintenance and testing of key aspects of the site. “The category 4 laboratories at IAH are very old and are well short of the standards expected of an internationally important laboratory handling such livestock-threatening pathogens as FMDV” (HSE, 2007, para. 13).

Insufficient financing for maintenance

Problems in the condition of buildings and in drainage at IAH had been recognized but, due to a shortage of and disputes over funding, the necessary maintenance work had not taken place:

Adequate funding has not been available to ensure the highest standards of safety for work on FMDV carried out at this aging facility. There had been concern for several years that the effluent pipes were old and needed replacing but, after much discussion between IAH, Merial and Defra, money had not been made available. (Spratt, 2007, para. 6, 33)

Suggested Areas for Amendment of and/or Addition to the International Standards

Advice on siting of laboratories and use of sentinel units

No particular advice was presented in the international standards on siting of laboratories working on Category 4 animal pathogens. Such advice may have questioned the appropriateness of siting such a laboratory within a few miles of farms on which livestock is kept. The 2008 version of the Terrestrial Manual advises that facilities be “in an isolated location” (Chapter 1.1.2.k.a), but further details would be helpful. This may include suggestions on the use of sentinel units to warn of releases. A general suggestion regarding the use of sentinels for animal health surveillance is included in the Terrestrial Code, and involves “the identification and regular testing of one or more animals of known health/immune status in a specified geographical location to detect the occurrence of disease” (Appendix 3.8.1). Placing sentinels between a laboratory site and surrounding farms may allow early identification and containment of disease releases before they reach the general animal population.

Guidance on shared facilities, conflicts of interest, and use of contractors

Communication and ownership issues can arise in shared facilities, hindering biosafety efforts—something noted in the Spratt Review (p. 8). The reports identified access between different areas of the site as a potential problem. The Spratt Review also highlighted potential conflicts of interest—Merial’s Director being its biosafety officer, and IAH’s main customer (Defra) being its regulator (pp. 5, 8). Guidance on these issues would be helpful. Additionally, guidance on use of contractors could be provided, advising, for example, on access controls and restrictions, recordkeeping, biosafety training, and disinfection procedures.

Standards for licensing and inspection

The Laboratory Biosafety Manual provides useful checklists for inspection, and the international standards recommend regular inspection and licensing of laboratories. Additional guidance may be useful—for example, specification of particular breaches for which licenses should be suspended or withdrawn.

Standards for status as a World Reference Laboratory

IAH Pirbright is designated as the OIE/Food and Agriculture Organization World Reference Laboratory for FMD and nine other animal diseases. The Terrestrial Manual states that these laboratories “have considerable experience in the operation of safe working practices and provision of appropriate facilities” (Chapter I.1.6). Questions arise about whether, particularly in regard to “appropriate facilities,” this description applied to IAH at the time of the outbreak, and what the standards were for assigning this status. The OIE’s Internal Rules for Reference Laboratories (OIE, 2006) shows that the experts at the laboratories and their competence are the basis for the approval of the designation. The achievement of core laboratory biosafety standards should also be considered, given that these laboratories are set out as examples of international excellence.

Conclusion

The analysis in this article strongly suggests that, had international laboratory biosafety and biosecurity standards been appropriately applied, the 2007 FMD outbreak in the UK would not have occurred. Application of international standards was lacking in several significant areas, which made the Pirbright site unsuitable for maximum containment work. Problems with national rules, procedures, and financing failed to identify and/or address these issues. Applying international standards may have been sufficient to prevent the outbreak, but there are some areas that could be dealt with more clearly in those standards.
References


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The Environmental Protection Agency has approved the first registration, or license, of an antimicrobial pesticide product to deactivate anthrax spores on hard surfaces. “Peridox with the Electrostatic Decontamination System” can decontaminate buildings, structures, vehicles, ships, aircraft, personal protective equipment, and other items infected with anthrax spores. Its use is limited to dry, precleaned, hard, nonporous surfaces.

EPA reviewed extensive data provided by the manufacturer, Clean Earth Technologies, to be sure that the product will be effective and not cause unreasonable adverse effects. EPA also reviewed the labeling of Peridox and associated training materials to ensure that they are consistent with EPA’s Pesticide Registration Notice 2008-2, which specifies the terms and conditions that would apply to anti-anthrax products.

The notice provides guidance to prospective applicants of antimicrobial products that claim to deactivate anthrax spores. The availability of such products will better prepare the United States to respond to anthrax incidents. The guidance assures that anthrax-related products are registered, bear appropriate labeling, and are effective when applied as directed. The use of anthrax-related products will be limited to federal on-scene coordinators, the U.S. military, and persons trained and certified competent by the manufacturer.

Peridox is the first pesticide registered to deactivate anthrax spores. EPA previously issued crisis exemptions allowing use of unregistered antimicrobial chemicals to clean buildings and any contents contaminated with anthrax spores.

Anthrax is a disease caused by *Bacillus anthracis*. Both humans and animals are susceptible. Anthrax, if untreated, can cause acute illness or death.

More information about the registration notice: www.epa.gov/pesticides/factsheets/chemicals/peridox-eds.html
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@mcw.edu or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

**Safety Overview of Techniques Involving miRNAs, siRNAs, and Other Small Regulatory RNAs**

Knowledge of miRNA function is rapidly expanding and new uses of miRNAs, siRNAs, and shRNAs as tools in biotechnology are emerging on a regular basis. MicroRNAs (miRNAs), small interfering RNAs (siRNAs), and short hairpin RNAs (shRNAs) are all small nonprotein-coding RNA molecules. While miRNAs are endogenous, siRNAs and shRNAs have been developed as tools to interact with mRNA (messenger RNA) to repress translation of mRNA into proteins. The miRNA field of study is relatively new with most work being done since 2000. As an evolving field, there are many unknowns about miRNAs including safety factors, but one clear take-away message is that miRNAs serve as important regulators of gene expression (Ying et al., 2008). Specifically, miRNAs have been proposed to regulate up to 90% of human genes through miRNA-guided RNA silencing (Perron & Provost, 2008).

miRNAs are transcribed in the nucleus as pri-miRNAs (primary microRNAs), then transported to the cytoplasm where the pri-miRNAs are processed to mature miRNAs which can then bind to mRNA targets to repress translation, alter mRNA cleavage, and promote mRNA decay/degradation. siRNAs and shRNAs are short RNAs manufactured to bind with mRNA to prevent or interfere with translation. piRNAs (piwi-associated RNAs) are a class of small RNAs associated with germline development (Klattenhoff & Theurkauf, 2008).

The central dogma of molecular biology is that DNA is transcribed into mRNA which is then translated into proteins. In addition, DNA copies are made (replication) and RNAs other than mRNA (transfer RNAs and ribosomal RNAs) are involved in translation. Some proteins serve as regulators of multiple processes involving nucleic acids such as transcription factors (regulators of gene expression). In reality, RNA molecules carry out a variety of functions in the cell. The delay in understanding miRNA function probably had a lot to do with the focus on the central dogma with the idea that the roles of endogenous RNAs were already understood. RNA playing a key role as a regulator of gene expression was a revolutionary idea.

miRNAs serve as functional counterparts of transcription factors—both are endogenous regulators of gene expression. While transcription factors regulate gene expression by interacting with DNA (up and down regulation of transcription), miRNAs regulate gene expression through interaction with mRNA (preventing translation, altering mRNA cleavage processes, and promoting mRNA degradation or decay). Use of siRNAs to inhibit translation pre-dated the understanding of the regulatory function of miRNAs. Studies targeting miRNA regulation have focused on RNA interference (down regulation of gene expression), but miRNAs and synthetic double-stranded RNAs (dsRNAs) can also act as RNA activators (up regulation of gene expression) (Pushparaj et al., 2008; Wu & Belasco, 2008).

miRNAs operate in a multitude of cellular and physiological processes including cell cycle regulation, embryogenesis, development of organ systems, differentiation, oncogenesis, tumor suppression, apoptosis, angiogenesis, toxicology, and immunity. Because of the wide-ranging effects of miRNA regulation, techniques to manipulate miRNAs are being successfully explored as treatment options for many diseases and disorders including cancer, infectious diseases, neurological disorders, metabolic disorders (e.g., diabetes), and cardiovascular and respiratory diseases.

Clinical oncology applications for miRNA technology are numerous since miRNAs can act as oncogenes and as tumor suppressor genes (Zhang et al., 2007) and play roles in cell cycle progression, apoptosis, angiogenesis, and metastasis (Fish & Srivastava, 2009; Gramantieri et al., 2008; Hermeking, 2007; Oulas et al., 2009; Tong et al., 2005).

Studies on the RNA-based immune system (small RNA-directed viral immunity) that is mechanistically related to RNA interference (Aliyari & Ding, 2009) have led to clinical applications for infectious disease prevention
siRNA technology has identified some safety concerns that are relevant to miRNA studies; these include cellular toxicity, extended stability, insertional mutagenesis if the delivery system is a viral vector that integrates, effects in non-targeted organs, and off-target effects with altered expression of genes other than the targeted gene. Delivery systems are being developed to minimize the cellular toxicity that occurs primarily due to the triggering of innate immune responses (Akinc et al., 2008; Behlke, 2006; Boudreau et al., 2008; Judge & McLachlan, 2008).

RNAs are inherently unstable. Unmodified siRNA has a half-life of less than an hour in human plasma, and circulating siRNAs are rapidly excreted by the kidneys due to their small size (Layzer et al., 2004; Li et al., 2006). Delivery systems are being developed that extend the life of the regulatory RNAs to expand their utility in treatment (Frieden & Orum, 2006), but from the safety standpoint, longer-lived RNA could lead to unintended deleterious effects. In addition, if the delivery system chosen is a viral vector, the potential infectivity hazard of replication-competent virus generation exists with the additional potential hazard of insertional mutagenesis if the viral vector is one that integrates into the host genome (Li et al., 2006). On a separate but related topic, traditional assessment of insertional mutagenesis risk focuses on insertional effects on protein-coding genes. With the growing understanding of miRNAs’ regulatory functions, assessment of insertional mutagenesis risk should also include the potential effects of vector insertion near non-coding sequences such as miRNAs.

Some in vivo studies with siRNA have demonstrated unwanted side effects due to the accumulation of siRNAs in organs other than the targeted one (Landen et al., 2005; Li et al., 2006). Several groups have successfully attempted to improve tissue-specific delivery of siRNAs by using either ligands for specific receptors or antibodies against cell surface markers (Li et al., 2006; Schifferlers et al., 2004; Zhang et al., 2003).

Specific miRNAs, like specific transcription factors, can regulate the expression of a number of different genes, and because complex pathways of regulation are involved, altering the regulation of a miRNA, like altering the regulation of a transcription factor, can have multiple consequences, some of which are unintended. Regulatory molecules are often part of a complex cascade of events, individual miRNAs can regulate a number of different mRNAs with effects on different cellular processes, and an exhaustive list of which mRNAs are regulated by any specific miRNA is often not known (Marquez & McCaffrey, 2008). miRNA-like target interactions can be triggered by extremely limited sequence homology resulting in off-target effects (Jackson et al., 2003; Li et al., 2006; Lin et al., 2005; Persengiev et al., 2004; Scacheri et al., 2004). Thorough studies of gene expression (in vitro and in vivo) with identification of the genes whose expression might be affected by specific miRNA techniques and with in vivo animal studies that identify potential unforeseen consequences are needed (Lewis, 2008). Such information potentially could lead to minimization of off-target effects through modification of experimental procedures. For example, small changes potentially could be made to the targeting sequence such that the desired mRNA continues to be targeted while targeting of other mRNAs is minimized (Jackson et al., 2006).

Use of techniques involving small regulatory RNAs such as miRNAs and siRNAs show great promise in the study of basic science questions as well as for therapeutic uses in a number of disorders including cancer. In the development and use of these techniques, safety concerns, particularly off-target effects and the consequences of greatly extending the life of small regulatory RNAs, should be considered in conducting risk assessments.

References

Antimicrobial Testing Program Web Page Now Available

EPA has posted an Antimicrobial Testing Program web page to inform the public of post-registration efficacy test results of disinfectant products on the market for use in hospitals and other public health facilities. EPA conducts post-registration testing of public health antimicrobial products to ensure that marketed products are effective against target microorganisms when used according to their label directions. The ATP has been testing hospital sterilants, disinfectants, and tuberculocides since 1991 to help ensure that products in the marketplace continue to meet stringent efficacy standards. Products bearing claims to control organisms that may pose a threat to human health—either directly or through transmission of disease-causing organisms on environmental surfaces—are considered public health-related antimicrobials and require efficacy data to support labeling claims and patterns of use.

Under the ATP, Agency and state contract laboratories conduct tests to verify an antimicrobial product’s effectiveness. The Agency has set the end of 2011 as the goal for completing the post-registration efficacy evaluation of the remaining hospital disinfectants and tuberculocides under the ATP. The web page at www.epa.gov/oppad001/antimicrobial-testing-program.html includes a list of products tested and their status. Updates to the product status list will be posted to the web site on a regular basis.
Ask the Experts

John H. Keene

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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@globalbiohazardtechnologies.com or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Protecting Surfaces in BSL-3 Suites and Animal Facilities—Are Work Surface Pads and Disinfectant or Sticky Floor Mats the Answer?

Question

Some laboratories have a mat with bleach solution at the entrance and exit of a BSL-3 suite. Is this procedure useful?

Answer

It is time for us all to understand that biocontainment laboratories are not contaminated with the agents that are being used in the laboratory, unless a catastrophic spill occurs outside a containment device. If the lab is always contaminated, then the people who are working in that lab should not be working there and the supervisor or PI should not be allowed to oversee the operation of the laboratory. In a biocontainment laboratory, all work with the agent is performed in the biosafety cabinet or other primary containment device and the floors should not be contaminated at all; therefore, shoes should not be contaminated and generally a mat soaked in bleach solution is not needed.

If there is a spill in the lab that requires cleanup (i.e., a spill outside of the containment device), then there should be a procedure for ensuring that lab personnel’s shoes are left in the lab and properly decontaminated prior to being removed from the lab. Even with a spill, a pad with disinfectant at the exit of the laboratory is not needed.

Let’s look at this practice from the standpoint of what we know about disinfectants and their action. In order to be effective, a given disinfectant must be in contact with the organisms of concern for an appropriate time, at an appropriate pH and temperature. A significant amount of extraneous organic matter should not be present, as the chemical disinfectant does not distinguish between the extraneous organic matter and the microorganisms. In addition, the disinfectant may lose activity over time. Finally, it is known that certain types of microorganisms can actually thrive in weak disinfectants (Gajadhar et al., 2003; Oie et al., 1996; Weber et al., 2007) and these pads, if not frequently changed or replaced, could actually serve as breeding grounds for organisms.

Given that a biocontainment lab worker’s shoes are unlikely to be contaminated, and considering the requirements for efficacy of the disinfectant and the considerable amount of extraneous organic matter on a worker’s shoes, it is suggested that such pads are a waste of money and it is highly unlikely that the disinfectant pad at the exit of a lab would be useful.

Question

Are these pads necessary at the entrance to biocontainment facilities, or useful in minimizing potential contamination in animal facilities?

Answer

To answer the first part of this question, let us understand that biocontainment laboratories are not clean rooms. Air flows into the biocontainment facility under and around the doors and there really is no need for them to be any “cleaner” than any other microbiology laboratory. Again, work is done in biosafety cabinets which, when working correctly and used by trained personnel, protect not only the worker and the environment, but also the work. Therefore, there is no need to “decontaminate” the shoes of personnel coming into the facility. In addition, given the information provided above for the “exit” pads, increased contamination could be spread through the improper use and maintenance of these pads.

The answer to the second part of the question regarding animal facilities follows the same logic with regard to potentially increasing contamination from improper use and maintenance. It is better to have dedicated shoes or shoe covers for personnel working with animals than it is to rely on “disinfectant pads.”

In writing this article I am reminded that a number of products on the market claim to be helpful in reducing the introduction of dirt, dust, microbes, etc. into supposedly clean areas. Sticky mats that are supposed to, and appear to, remove dirt and microbes from the feet of personnel entering operating rooms and clean rooms come to mind. Daschner and co-workers stated that “useless and unproven methods in hospital infection
control are: environmental cultures, routine cultures of personnel, routine air sampling, fogging and spraying of disinfectants, UV lights, plastic shoe covers, routine floor disinfection, disinfection or sticky mats,..." (Daschner et al., 1987). While these devices appear to be removing dust and dirt from the shoes of personnel, the fact is that once someone has walked on the mat and deposited his or her contamination, the next person stepping on the mat in the same place simply picks up the residue left behind by the first person. To be effective, these mats would have to be removed/replaced after each person enters or exits the area and that does not happen. Remember, if a salesman approaches you with a new and better way to protect your personnel and it looks too good to be true, ask for the data that definitively proves the efficacy of the product. If they can’t be produced, don’t buy the product.

**Question**
Should absorbent pads be used on the work surface of BSCs in BSL-3 laboratories and if so, why?

**Answer**
This is one of those interesting procedures that seem to have been introduced into the laboratory and no one seems to know when or how it started and why it should be done except for the fact that “we’ve always done it this way.” In fact, the use of absorbent pads actually pre-dates biosafety cabinets and is probably, if done correctly, a very good practice to minimize the potential for aerosolization of spilled materials.

In October 1952, even before I started working in microbiology, Raymond Anderson and his coworkers at Camp Detrick (now Fort Detrick) in Frederick, Maryland recognized that certain procedures used in the microbiological laboratories might result in the formation of aerosols and the subsequent infection of workers in the laboratory.

In an article entitled “Potential Infectious Hazards of Common Bacteriological Techniques” published in the *Journal of Bacteriology*, Anderson described their research on the potential for infectious aerosol formation due to common laboratory procedures and accidents (Anderson et al., 1952). Among the experiments performed, they examined different procedures that might cause a spill on various laboratory surfaces to determine if aerosols were released. I’ll not bore you with all of the results because I encourage you to read this interesting and historic article for yourself at www.pubmedcentral.nih.gov/articlerender.fcgi?artid=169381&log$=activity.

They demonstrated that when certain procedures that resulted in droplets falling on surfaces were performed, less aerosol was created if the droplets fell on damp absorbent towels placed on the work surface. More specifically, they demonstrated that the absorbent material had to be dampened with phenol to significantly reduce the potential for aerosolization. Dry towels produced almost as much aerosol as the solid surface; however, paper towels, wrapping paper, and towels wet with phenol did not result in significant aerosolization. Anderson and his coworkers, while noting this result, did not postulate a reason for the differences.

So, the answer to the question is that not only should we probably be using absorbent pads on the work surfaces of BSCs in BSL-3 labs, but we should also use these pads on all work surfaces for all work done with infectious agents in microbiology laboratories. One must remember, however, that these pads should be removed following the completion of the work and disposed of in a manner consistent with other potentially contaminated materials in the laboratory. In addition, remember that, at least for the absorbent towels, dry towels did not seem to be as effective as damp towels. Perhaps someone should do some research with modern absorbent pads to see if they really work as well as we hope they do. Another argument for applied biosafety research.

**References**


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**Example**

**Financial Disclosures:** None reported.

**Example**

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This Disclosure Statement is posted on the online journal submission site at: www.x-cd.com/absa/article.cfm. Questions should be sent via e-mail to the Production Editor, Karen Savage, at karen@absaoffice.org.

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Recombinant DNA News

Clonal Population of Cells Detected in a Clinical Human Gene Transfer Trial Using Lentiviral Vector

The National Institutes of Health Office of Biotechnology Activities (OBA) has been informed that a “relative clonal dominance” was detected during follow-up of a subject who is participating in a French human gene transfer trial being conducted for individuals with β-Thalassemia Major and Sickle Cell Anemia. The clinical trial, sponsored by Genetix France, used hematopoietic stem cells transduced by a self inactivating (SIN) HIV-1 lentiviral vector containing the gene for β-globin under the control of the β-globin promoter. The subject received the gene modified cells in June 2007.

This clonal dominance appears to result from the integration of the vector in the gene encoding for the HMGA2 protein, which is associated with both benign and malignant tumors. The clone however has been stable for five months and the subject remains in good health. In fact, although the subject required almost monthly blood transfusions during the 11 months prior to the gene transfer intervention, the subject has not since required a blood transfusion.

The investigators involved in this trial will be performing further studies to evaluate the consequences of this integration and its capacity to proliferate. Until these studies are completed and another review is performed by the French Medicine Agency, AFSSAPS, no other subjects in this study will receive the gene modified cells.

OBA has sent a memorandum to inform OBA-registered investigators who are using or propose to use lentiviral or retroviral vectors in hematopoietic or other stem cells. OBA is currently seeking additional information regarding this event such as the specifics of the vector used, the dose of the cells, transduction conditions, and the clinical course of the first subject treated in this trial. OBA will organize a discussion at a meeting of the NIH Recombinant DNA Advisory Committee (RAC) when this information is available.
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