Scientists call moratorium on study of deadly bird flu

Those who work with the H5N1 virus announce a 60-day voluntary halt to explain the benefits of research and the measures to minimize risks to the public.

January 20, 2012 | By Amina Khan, Los Angeles Times

In an almost unheard-of move, scientists who study the deadly H5N1 bird flu announced a 60-day voluntary moratorium on studying the virus to allow time “to clearly explain the benefits of this important research and the measures taken to minimize its possible risks.”

The statement, released Friday by the journals Science and Nature, comes soon after federal officials had asked the journals and two research teams to withhold details of experiments that showed the virus can be coaxed to a form that passes readily through the air from mammal to mammal.

Update On Risks At Animal Disease Lab Out Tomorrow

BY LAURA ZIEGLER | KCUR.ORG, KANSAS CITY PUBLIC MEDIA

In a highly anticipated announcement, the National Academies of Science said that it will release a congressionally mandated report on risk associated with the National Bio and Agro-Defense Facility (NBAF) this Friday.

Stalled congressional funding is believed to be linked to the results of this report.

The animal disease laboratory, under construction in Manhattan, Kan., is a project of the Department of Homeland Security.

This latest report is actually an update of the department’s original risk assessment, which was predicated on misguided assumptions and methodology, NAS said. It will deal with whether DHS has adequately addressed risks identified in the original report.

California Researcher Dies From Infection He Studied

By KATIE MOISSE (@katiemoissee)
May 4, 2012 | ABC News

The death of a 35-year-old researcher studying a sinister strain of bacteria has highlighted the hazards of infectious disease research.

The man, whose name has not been released, died Saturday at the San Francisco VA Medical Center from a meningococcal infection he may have acquired while working at the hospital the day before.

Headache, fever and chills set in just two hours after the researcher left the lab where he was helping to develop a vaccine for Neisseria meningitdis, a bug that causes life-threatening blood infections and meningitis.

61084 Federal Register / Vol. 77, No. 194 / Friday, October 5, 2012 / Rules and Regulations

DEPARTMENT OF HEALTH AND HUMAN SERVICES
[Docket No. CDC--2011--0012]
42 CFR Part 73
RIN 0929-AA34
Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review

AGENCY: Centers for Disease Control and Prevention (CDC), Department of Health and Human Services (HHS).

ACTION: Final rule.

SUMMARY: In accordance with the Public Health Security and Bioterrorism Preparedness and Response Act of 2002, the Centers for Disease Control and Prevention (CDC) located within the Department of Health and Human Services (HHS) has reviewed the list of biological agents and toxins that have the potential to pose a severe threat to public health and safety and is...

54584 Federal Register / Vol. 77, No. 172 / Wednesday, September 5, 2012 / Notices

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Final Action Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

SUMMARY: On March 4, 2009, the National Institutes of Health (NIH) Office of Biotechnology Activities, Office of Science Policy (NIH/OE) published a proposal in the Federal Register (74 FR 9411) to revise the NIH Guidelines in two regards. The first was to address biosafety considerations for research with synthetic nucleic acids. The proposal modified the scope of the NIH Guidelines specifically to cover certain basic and clinical research with nucleic acid molecules created solely by synthetic means. The second proposed revision was to modify the criteria for determining whether an experiment to introduce drug resistance into a microorganism must be reviewed by the Recombinant DNA Advisory Committee (RAC) and approved by the NIH Director (as a Major Action under Section III-A-1-a of the NIH Guidelines). Comments...
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About the Cover

Biosafety has been featured prominently in the news in 2012 and has been pivotal in decisions being made regarding the conduct of research and institution of a moratorium on research with highly pathogenic avian influenza, as well as the decision whether to construct the National Bio and Agro-Defense Facility as designed, or a down-sized design. Sadly, we still read about reports of preventable laboratory acquired illnesses, some resulting in fatality. Organizations that comply with the Select Agent Regulations or the Research Involving Recombinant DNA Molecules guidelines saw changes in the Federal Register that could impact laboratory operations. These are only a few examples where biosafety in the news has influenced the research environment. Information on the cover can be accessed at:

http://www.selectagents.gov/resources/CDC%20Select%20Agent%20Biennial%20Review%20Final%20Rule%202010%2005%202012.pdf

Calendar of Events

February 4-7, 2013
USDA ARS 2nd International Biosafety and Biocontainment Symposium
Hilton Alexandria Mark Center, Alexandria, Virginia
Contact: Phone: 1-866-425-1385; E-mail: info@absa.org; www.absa.org

February 24—March 1, 2013
ABSA Principles & Practices of Biosafety (PPB)
Embassy Suites Orlando—Lake Buena Vista South, Kissimmee, Florida
Contact: Phone: 1-866-425-1385; E-mail: info@absa.org; www.absa.org

April 15-18, 2013
6th Annual Leadership Institute
Managing Unmanageable Situations: Management Techniques and Strategies for Biosafety Professionals
Charleston Marriott, Charleston, South Carolina
Contact: www.absa.org/eduleadership.html

July 21-26, 2013
ABSA Principles & Practices of Biosafety (PPB)
Embassy Suites Portland—Downtown, Portland, Oregon
Contact: Phone: 1-866-425-1385; E-mail: info@absa.org; www.absa.org

October 17-23, 2013
ABSA 56th Annual Biological Safety Conference
Sheraton Crown Center, Kansas City, Missouri
Contact: Phone: 1-866-425-1385; E-mail: info@absa.org; www.absa.org

Coccidioidomycosis: Focus on Occupational Health Issues

The Division of Environmental and Occupational Disease Control in the California Department of Public Health has developed free online continuing medical education (CME) courses on a variety of occupational and environmental medicine topics. This 1-hour course provides information on the epidemiology, diagnosis, and treatment of coccidioidomycosis; highlights workers at risk for occupational coccidioidomycosis; and describes measures to prevent its occurrence. For more information, visit www.cdph.ca.gov/programs/deodec/Pages/CMEcourses.aspx
Applied Biosafety: Journal of the American Biological Safety Association (ISSN 1535-6760) is published quarterly by the American Biological Safety Association (ABSA). ABSA members receive the journal as a benefit of membership. An additional annual subscription for members is $60. Nonmembers and institutions/libraries may subscribe at the annual rates of $110 and $145 respectively. Single issue rates are as follows: members $18; nonmembers $28; and institutions/libraries $35. Annual nonmember electronic subscription is $35.00.

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Online Journal Submission
Applied Biosafety: Journal of the American Biological Safety Association is a peer-reviewed, scientific journal committed to promoting global biosafety awareness and best practices to prevent occupational exposures and adverse environmental impacts related to biohazardous releases. The online submission site for Applied Biosafety is available at www.x-cd.com/absa/article.cfm. For more information or questions, please contact the Production Editor, Karen D. Savage, at the ABSA Office at 1-866-425-1385 (toll free) or 847-949-1517 or via e-mail at karen@absaoffice.org.

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Guest Editorial

Surviving Biosafety: Coping with Occupational Stressors of Serving the Profession

Sean G. Kaufman*

Emory University, Atlanta, Georgia

The Federal Select Agent Program (FSAP) recently released new guidelines for organizations working with select agents. These guidelines, along with recommendations in Biosafety in Microbiological and Biomedical Laboratories (BMBL) (U.S. Department of Health and Human Services, 2009) and other safety references, are simply words on paper which aim to minimize overall risk associated with biological scientific research. Biosafety officers play an instrumental role in biological risk mitigation by identifying risks, assessing risks, developing organizational plans to manage these risks, and communicating the risk/benefit equation to those expected to comply with these guidelines. Alone, these guidelines do not produce consistent behavioral practices among the workforce. Because behavioral inconsistencies may lead to a variety of outcomes which may not be safe, biosafety officers may hold themselves or be assigned accountability should an unsafe incident occur within a biocontainment facility.

The burden of integrating a guideline into an organization may fall solely on the biosafety officer. This burden may lead to several occupational stressors including but not limited to high workloads, long hours, limited resources, immediate deadlines, feeling undervalued, and lack of task control. A study by the American Psychological Association found many Americans listed the causes of work stress to include low salaries (46%), lack of opportunities for growth and advancement (41%), heavy workloads (41%), long hours (37%), and unclear job expectations (35%) (APA, 2012). These occupational effects may have a profound impact on overall safety within organizations. A biosafety officer who is exhausted, feels unmotivated, and lacks needed resources may not have the energy or cognitive ability to minimize the risks associated with daily laboratory activities. This not only puts the laboratory staff at increased risk but could also pose an increased risk to the organization’s overall reputation, research agenda, and overall liability.

In October 2012, Emory University hosted a 30-minute teleconference as part of its monthly Lunch Break Series titled “Surviving Biosafety: Coping with the Occupational Effects of Serving the Profession.” During this teleconference, strategies for coping with the seven stressors listed above were presented and discussed. In some instances, stress on the job may be a good thing; however, stress has also been directly linked to employee withdrawal behaviors including absenteeism and turnover (Gupta & Beehr, 1979). Feelings of inadequate control over one’s work and frustrated hopes and expectations seem to be independent causes of burnout, a term that describes a condition of professional exhaustion. This exhaustion may lead to low levels of satisfaction with the overall job, impacting interpersonal and family relationships and leading to a negative attitude toward life in general (A lacovides, 2003). The following are descriptions and recommendations for dealing with common workplace stressors, some of which biosafety officers may be experiencing.

High Workloads

A biosafety officer’s workload is the perception of work demands placed on him or her during any given day. Biosafety officers who feel they have more work than they can accomplish in one day are experiencing the stress of a high workload. High workloads increase emotional distress both in the workplace and at home. Individuals experiencing high workloads also evidence higher blood pressure rates which significantly impact overall health (Ilies, 2010).

Recommendations for dealing with high workloads include delegating tasks to others and managing tasks more efficiently. For example, a biosafety officer may assess the amount of time he or she spends responding to e-mails and decide to reduce this activity by dedicating specific daily time slots to e-mails, thus allowing more time to engage in other activities and accomplish other tasks. Taking breaks during the day to re-energize is another strategy to better manage high workloads. This strategy is not only needed, but also has been shown to increase workplace productivity.

Limited Resources

According to the job demands-resources (JD-R) model (Demerouti et al., 2001), work conditions can be divided into two broad categories: job demands and job resources. Job demands are strongly related to worker exhaustion (aka, burnout), whereas job resources are primarily related to work disengagement. High workloads lead to worker exhaustion, while limited resources lead to worker disengagement. Biosafety officers who are disengaged and not fully committed to their workplace may exhibit low morale and decreases in overall work performance.
Dealing with limited resources begins with differentiating ideal, preferential, and acceptable safety practices. Each level requires a different quantity of resources; however, an evaluation may show that there will be little variation in the overall outcome. In that case, diverting resources away from an “ideal” level may be appropriate. Another strategy to increase resource allocation is to conduct workplace assessments and solicit feedback on the resources needed from the population the biosafety officer serves. Having others state what is needed provides additional weight to biosafety requests and may increase the likelihood of gaining additional resources.

**Lack of Control with Immediate Deadlines**

Stress may begin to occur when a biosafety officer feels as though he or she has no control over workplace responsibilities and activities. The sense of control is reduced when an assignment with an immediate deadline is imposed, and this impacts overall employee motivation. Externally imposed deadlines lower employee interest and motivation to address workplace tasks (Amabile et al., 1976). Low interest and motivation levels among biosafety officers may lead to unsafe work environments and increases in organizational risk.

The primary recommendation for dealing with immediate deadlines is to establish healthy boundaries. Biosafety officers must be able to communicate what can and cannot be done by a specific deadline. If one task takes priority over another, the biosafety officer must communicate the impact of the new deadline on existing responsibilities rather than accept the additional burden without explanation of what it means to the workplace. Failure to establish healthy boundaries may lead to burnout, incomplete tasks, poorly completed tasks, and general safety oversights.

**Feeling Undervalued**

A recent study by the American Psychological Association (APA, 2012) indicated that among individuals who felt undervalued at work, only 33% said they were motivated to do their best at work and only 38% reported feeling engaged. The study also indicated many reasons for feeling undervalued in the workplace including not being involved in the decision-making process, being less satisfied with potential growth and advancement opportunities, and restrictions on the ability to utilize flexible work options. If a biosafety officer feels undervalued, leading to decreased levels of motivation and workplace engagement, organizational risks may increase over time.

Unfortunately, there may not be many opportunities for growth and advancement for biosafety officers. Additionally, the need for biosafety officers to be present and available at all times may also minimize their ability to have flexible work options. This poses a challenge to biosafety officers, as feeling valued must be influenced more by intrinsic rather than extrinsic factors. Thus, biosafety officers should do their best to remember why they became a biosafety officer in the first place. They must identify what activities make them feel valued and utilize these activities to motivate and engage themselves back into the workforce. Communicating his or her sense of being undervalued to leadership can be considered; however, this communication should occur only after the biosafety officer has identified activities that would increase his or her sense of professional value so that requests to participate in these activities can be considered during or following the discussion.

**Long Hours**

A recent study published in the *Journal of Epidemiology* (Virtanen et al., 2012) has shown that a combination of stress, raised blood pressure, and unhealthy diets stemming from long working hours (>8 hours) may be the causes of serious health problems. Furthermore, individuals working long hours are at a 40% increased rate of suffering from coronary heart disease. Additionally, Sparks (1977) conducted a review of the literature on working hours and health and performed meta-analyses on 21 study samples. Results indicated positive mean correlations among overall health, physiological and psychological health, and hours of work.

A biosafety officer who works long hours, every day of every week, may have reduced physiological and psychological health, as well as lower engagement and motivation levels. To minimize long hours, biosafety officers must prepare as though they are running a marathon instead of a sprint and establish healthy boundaries that include walking away from work after 8 hours. Moreover, the biosafety officer’s positive impact on the organization may not be appreciated in a day, a week, a month, or even a year. Since recognition may come slowly, biosafety officers must remain healthy, engaged, and motivated. To do this, they must be able to walk away at the end of the day, go home to reenergize, and return to work ready to continue the race.

Regardless of the profession, workplace stressors have a profound impact on personal health and quality of life. Biosafety officers should consider the recommendations discussed above to minimize workplace stress. However, implementing these changes may lead to initial increases in stress (as change usually does), and as a result, activities to reduce stress may not even be considered. The decision to implement any recommendation/change is a personal one that may be influenced by unknown environmental factors; however, the greatest factor of influence for change is always the personal choice to change.

An old story of a wise man and a young cow herder sums this up best: Once there was a little boy who raised cows. He would herd them up a mountain every day and at the end of the day come back, tie them up, and leave them overnight. One day, as he was tying up the cows, he noticed he did not have a rope for one of the cows and be-
came concerned the cow would walk away during the night. The young cowherder asked a wise man what he should do. The wise man told him to go back to the cow, pretend to tie up the cow, and make sure the cow watches you do this. The little boy listened to the wise man and did as he was instructed. The next morning, to the little boy’s amazement, the cow was standing right where he had left it. He untied all the other cows but the cow he had pretended to tie up would not budge. He did everything he could to move the cow, but the cow simply would not move. He returned to the wise man and asked what he should do. The wise man told him, “Untie the cow!” So the little boy returned, pretended to untie the cow, and watched as the cow returned to the herd.

The reason change may not occur within an individual is an “invisible rope” — a limitation that one believes exists and yet it truly does not. By applying the model for biological risk mitigation to stress mitigation, biosafety officers can clearly identify the risks associated with the stressors discussed above. Then they must assess the impact of these stressors on their personal and professional lives, determine effective stress management activities and communicate with those they serve. Identify, assess, manage, and communicate — these are not only biological risk mitigation strategies but also a biosafety officer stress reductive strategy as well.

Acknowledgments

This article was developed from an Emory University monthly Lunch Break Series titled “Surviving Biosafety.” I would like to acknowledge Dr. Sherry S. Bohn from the National Biodefense Analysis and Countermeasures Center (NBACC) for inspiring the initial Lunch Break Series and for her commitment to the profession of biosafety.

*Correspondence should be addressed to Sean Kaufman at sgkaufm@emory.edu.

References


Potential Pandemic Influenza A/H5N1 Research

The risks and benefits of potentially pandemic Influenza A/H5N1 research are hotly debated, and the issues are highlighted in the open-access articles below. These viewpoints deserve your careful consideration.


Richard D. Henkel*, Thomas Miller, and Robbin S. Weyant

Centers for Disease Control and Prevention, Atlanta, Georgia

Abstract

The APHIS/CDC Form 3, Report of Theft, Loss or Release of Select Agents and Toxins (TLR Incident report) is the mechanism by which the theft, loss or release of a biological select agent and toxin (BSAT) is reported to the United States Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS) or Health and Human Services (HHS)/Center for Disease Control and Prevention (CDC). A total of seven hundred and twenty seven (727) TLR Incident Reports were received by CDC between 2004 and 2010. Based on information contained in these reports, there were:

- No reports of the theft of any BSATs,
- One confirmed loss of a BSAT occurred during shipment out of 3412 transfers of BSAT conducted during that time period.
- Eleven total laboratory acquired infections (LAIs) associated with BSAT releases reported to CDC between 2004 and 2010, in an average annual population of approximately 10,000 individuals with approved access to BSATs. No fatalities resulted from these infections and there were no reported cases of secondary transmission to other humans.
- Annual increases in the number of TLR reports submitted to CDC from 16 reports in 2004 to 269 reports in 2010.
- Approximately half of the reports were submitted by entities registered with CDC and approved to possess BSATs (59%) with slightly less than half submitted by entities exempt from registration and inspection (41%), (primarily diagnostic laboratories). In 2009 and 2010, the number of TLR incident reports from exempt entities 104 and 148, respectively) exceeded those submitted by registered entities (88 & 96, respectively). The majority of TLR Incident Reports from registered entities involved Biosafety Level 3 (BSL-3) laboratories. Most reports from exempt entities involved Biosafety Level 2 (BSL-2) laboratories.
- The majority of reports from both registered and exempt entities involved bacterial agents.

These results show that the Federal Select Agent Program has been successful in implementing a monitoring program, increasing compliance of registered and exempt laboratories (as evidenced by increasing numbers of reports) and effective investigation that identified some LAIs, and resolved other loss & release reports to determine that biosafety and security in U.S. labs is being sustained.

Introduction

After the 2001 anthrax attacks in the United States, Congress recognized the growing threat posed by BSATs as potential weapons of mass destruction. Congress enacted the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and the Agricultural Bioterrorism Protection Act of 2002 expanded the authority of the federal government to regulate the possession, use or transfer of agents and toxins that have the potential to pose a severe threat to public health and safety, to animal health and animal products, or to plant health and plant products (biological select agent and toxin, BSAT) (42 Code of Federal Regulations [CFR] Part 73, 9 CFR Part 121, and 7 CFR Part 331, respectively). These regulations gave the federal government the power to specify conditions that must be met by all individuals, private and public organizations, academic institutions and government agencies in order to be lawfully in possession of biological select agents and toxins (BSAT). The enforcement of these regulations has been delegated to CDC and APHIS by the HHS and USDA Secretaries, respectively. The Division of Select Agents and Toxins (DSAT) at CDC has been assigned the responsibility for oversight of the BSAT regulations found in 42 CFR Part 73.

One of the key elements of the select agent regulations is the requirement imposed on all entities (e.g., laboratories or individuals) to immediately report any theft, loss or release (TLR) of BSATs to either CDC or APHIS (42 CFR § 73.19). This applies to entities that are required to register with CDC or APHIS (e.g., those possessing, using, or transferring select agents) and undergo inspections as well as those exempted under 42 CFR § 73.5. Exempt entities primarily consist of diagnostic laboratories that are not registered with CDC or APHIS to possess BSATs, but may encounter them in their routine diagnostic activities. Both registered and exempt entities must also submit a more detailed report within seven calendar days following the occurrence or recognition of a TLR incident. These requirements allow CDC or APHIS to ensure that all TLR incidents are reported to law enforcement authorities when necessary, to assess risk to public health, worker safety and animal or plant resources, and to respond with appropriate actions as needed.
In the current manuscript, we present data reported to CDC from 2004-2010 following implementation of a nationwide program for monitoring the potential theft, loss, or release of BSATs.

**Materials and Methods**

**TLR Incident Reporting Requirements**—Reporting requirements for all entities in possession of BSATs are found in 42 CFR § 73.19 entitled “Notification of theft, loss, or release.” Specifically, “Upon discovery of the theft or loss of a select agent or toxin, an individual or entity must immediately notify CDC or APHIS and appropriate Federal, State, or local law enforcement agencies. Thefts or losses must be reported even if the select agent or toxin is subsequently recovered or the responsible parties are identified” (42 CFR 73.19[a]). In addition, “A completed APHIS/CDC Form 3 must be submitted within seven calendar days” (42 CFR 73.19[a][2]).

In addition, “Upon discovery of a release of a select agent or toxin causing occupational exposure or release of a select agent or toxin outside of the primary barriers of the biocontainment area, an individual or entity must immediately notify CDC or APHIS” (42 CFR 73.19[b]). “A completed APHIS/CDC Form 3 must be submitted within seven calendar days” (42 CFR 73.19[b][2]).

The 2008 Federal Select Agent guidance document (Select Agents and Toxins Theft, Loss or Release Information Document) defines an “occupational exposure” as “any event which results in any person in a registered facility or lab not being appropriately protected in the presence of an agent or toxin.” This may include reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potential infectious materials that may result from the performance of a person’s duties. For example, a sharps injury from a needle being used in select agent or toxin work would be considered an occupational exposure. This definition for any occupational exposure is derived largely from the Occupational Safety and Health Administration (OSHA) regulation 29 CFR § 1910.1030 (Bloodborne pathogens).

**Event data collection and processing**—Information contained in APHIS/CDC Form 3, Report of Theft, Loss or Release of Select Agents and Toxins submitted by reporting entities to CDC from 2004 through 2010 was used in this analysis. Information derived from these forms and other communications that is relevant to this manuscript includes: 1) the reporting entity type (registered, exempt), the biocontainment facility type, the agent, and the event type (theft, loss or release).

**Disposition of reports.** The first response by CDC after initial notification of a TLR incident involves an immediate assessment of the public health risk posed by the incident. The initial notification could be sent immediately or within 7 days.

The second phase of the TLR incident report disposition process involves a number of steps including:

1) **Data collection and processing**—CDC collects and reviews all information provided by an entity in the initial report of the event, information provided in the Incident report form, information collected through verbal communications and written correspondence during the post-event evaluation process, and other information obtained during inspections and investigation of the event. If appropriate, referrals to law enforcement and/or consultations with subject matter experts are made at this time.

2) **Review and evaluation**—CDC conducts a review of factors that may have contributed to the occurrence of the TLR event, such as deficiencies in training, equipment malfunctions or inadequacies in safety procedures. These evaluations include attempts to identify steps that can be taken to reduce the likelihood of a recurrence of the event, such as improved engineering or work practice controls.

3) **Follow-up and report closure**—CDC requests confirmation that appropriate medical evaluation and interventions are being provided for potentially exposed workers. In addition, CDC requests periodic reports of potentially exposed worker health status, when necessary, and that any adverse change in the health status of the potentially exposed workers related to this event is immediately reported to CDC. CDC also provides contact information for subject matter experts in other organizational units at CDC to entities and occupational health providers for assistance, if requested.

4) CDC also requests written assurance, when appropriate, that all specimens and samples that may contain BSATs are either properly destroyed, secured or transferred to another entity approved for storage of the BSATs. Depending upon the seriousness of the incident, CDC may perform onsite visits to enhance incident evaluation and fact-finding or to verify the application of remediation procedures.

**Data analysis**—Information received by CDC from entities reporting TLR events was collected from the sources discussed above and compiled into an Excel spreadsheet. Information regarding the type of agent, laboratory type, activities associated with the TLR event, entity type, and other pertinent facts were analyzed to identify patterns and trends that may be useful for improving laboratory safety and security. For the calculation of rates of reports per 1000 workers, the total number of workers with approved access to select agents was used as a denominator. For calculation of reports per 100 registered entities, the total number of registered entities obtained from the National Select Agent Registry (NSAR) data base was used as a denominator.

The rate of report submissions per 100 registered entities or per 1000 workers with security risk assessment (SRA) approval was determined for each year between 2004 and 2010. The rate of report submissions per 100 registered entities or per 1000 workers was calculated by dividing the number of each type of report by the number of entities with completed registration documentation (i.e., registered entities) x 100 or by the number of workers with
approved security risk assessments at registered facilities x 1000, respectively.

The total number of BSAT shipments approved by CDC was determined by counting the number of approved requests for transfers.

Results

A total of 727 TLR Reports were received by CDC between 2004 and 2010 (Table 1). During this time period, there were no thefts of BSATs reported to CDC. Of the 727 reports received, 88 (12%) were loss reports and 639 (88%) were release reports. The final disposition of BSATs described in the 88 loss reports was reconciled in all but one report. In this particular case, a shipment of Coccioidioides immitis was lost during transit. An investigation of this incident led by the FBI concluded that this shipment was apparently destroyed during processing at a commercial shipping facility in the United States.

There were 11 laboratory acquired infections (LAIs) that resulted from the incidents described in the 639 release reports received between 2004-2010. These LAIs were associated with exposures to Brucella melitensis (4 cases), B. suis (2 cases), Francisella tularensis (4 cases) and Coccioidioides immitis/posadasii (1 case) (Kaye, 2005, Mattar et al., 2009). Our follow up investigations showed that there were no fatalities associated with any of these LAIs, nor were there any confirmed instances of a secondary infection to any other person involving these select agents, either by direct exposure to an infected laboratory worker or from an environmental release reported to CDC.

The annual distribution of TLR Reports submitted between 2004 and 2010 is shown in Table 1. The number of reports received annually ranged from 16 in 2004 to 269 in 2010. There was a substantial increase in the total number of reports each year throughout the entire time period. Approximately 70% (62 of 88) of all loss reports were submitted between 2007 and 2010, and approximately 94% (601 of 639) of all release reports were received during this same 4-year period. The number of release reports from registered entities increased by nearly three-fold in 2007 from the previous year (Figure 1). Additional increases occurred each year successively thereafter from 47 reports in 2007 to 80 in 2008, to 88 reports in 2009 and to 96 reports in 2010. There was also an increase in the number of loss reports from registered entities from 5 reports in 2007 to 25 in 2010 but the number of these reports fluctuated during the 7 year period.

The rate of report submissions per 100 registered entities is shown in Figure 2 (left axis) along with the number of registered entities (right axis) for each year. The number of entities registered to possess BSATs during this time period ranged from a low of 136 in 2004 to a maximum of 327 registered entities in 2005.

The rate of release reports ranged from 2.8 reports in 2005 to 29.6 reports per 100 registered entities in 2010. The rate of release report submissions tripled between 2006 and 2007 and increased by nearly 60% again in 2008. The rate of increase in release reports subsided to less than 10% in 2009 and 2010. The rate of loss report submissions de-

Table 1
Number of BSAT loss and release reports received by CDC between 2004 -2010.

<table>
<thead>
<tr>
<th></th>
<th>Loss</th>
<th>( % )</th>
<th>Release</th>
<th>( % )</th>
<th>Total</th>
<th>( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>#</td>
<td>(%)</td>
<td>#</td>
<td>(%)</td>
<td>#</td>
<td>(%)</td>
</tr>
<tr>
<td>2004</td>
<td>8</td>
<td>(9.1)</td>
<td>8</td>
<td>(1.3)</td>
<td>16</td>
<td>(2.2)</td>
</tr>
<tr>
<td>2005</td>
<td>12</td>
<td>(13.6)</td>
<td>9</td>
<td>(1.4)</td>
<td>21</td>
<td>(2.9)</td>
</tr>
<tr>
<td>2006</td>
<td>6</td>
<td>(6.8)</td>
<td>21</td>
<td>(3.3)</td>
<td>27</td>
<td>(3.7)</td>
</tr>
<tr>
<td>2007</td>
<td>5</td>
<td>(5.7)</td>
<td>52</td>
<td>(8.1)</td>
<td>57</td>
<td>(7.8)</td>
</tr>
<tr>
<td>2008</td>
<td>15</td>
<td>(17.0)</td>
<td>113</td>
<td>(17.7)</td>
<td>128</td>
<td>(17.6)</td>
</tr>
<tr>
<td>2009</td>
<td>17</td>
<td>(19.3)</td>
<td>192</td>
<td>(30.0)</td>
<td>209</td>
<td>(28.7)</td>
</tr>
<tr>
<td>2010</td>
<td>25</td>
<td>(28.4)</td>
<td>244</td>
<td>(38.9)</td>
<td>269</td>
<td>(37.0)</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>(100)</td>
<td>639</td>
<td>(100)</td>
<td>727</td>
<td>(100)</td>
</tr>
<tr>
<td>Annual Average</td>
<td>13</td>
<td></td>
<td>91</td>
<td></td>
<td>104</td>
<td></td>
</tr>
</tbody>
</table>

* = There were no thefts reported between 2004-2010
** = percent of total reports in category for 2004-2010
**Figure 1**
Annual numbers of BSAT release event reports from registered and exempt entities between 2004 and 2010. A comparison between the number of reports received from registered (dark bars) and exempt (light bars) entities is shown graphically for each year between 2004 and 2010. The actual number of reports submitted by both types of entities each year is also shown above the bars. There were no reports submitted by exempt entities in 2004 and 2005.

**Figure 2**
Annual rates of BSAT reports of loss and release events submitted to CDC per 100 registered entities, 2004-2010. The rate of reports of loss events per 100 registered entities between 2004 and 2010 is graphically illustrated using lines connecting solid squares. The annual rate of release event reports is indicated using open circles. The scale for both loss and release report rates are shown on the left side of the graph. The number of entities registered with CDC to possess BSATs each year that was used to calculate the rates shown in this graph is also indicated for each year using “X” symbols. The reference scale for the number of registered entities is shown on the right side of the graph. The numerical values for loss and release rates as well as the number of registered entities are shown above each symbol.
clined from 5.9 in 2004 to 1.7 in 2007 but then increased to 7.7 reports per 100 registered entities in 2010.

The rate of report submissions per 1000 workers with security risk assessment (SRA) approval between 2004 and 2010 is shown in Figure 3 (left axis) along with the number of SRA approved workers for each year (right axis). The number of SRA approved workers at registered entities ranged between a low of 8,185 workers in 2004 to a maximum of 10,979 workers in 2009. There was a small (< 4%) decline in 2010 to 10,639 approved workers. On average, there were 9,768 SRA approved workers annually between 2004 and 2010. The rate of release report submissions ranged from 1 report in 2004 to 9 reports per 1000 workers in 2010. Most of this increase occurred in 2007 (+167%) and 2008 (+54%). Additional annual increases of approximately 8% and 9% in this rate were observed in 2009 and 2010, respectively. The rate of loss report submissions fluctuated between 0.6 in 2006 and 2.3 per 1000 workers in 2010.

Prior to 2006, there were no TLR reports received from exempt entities. The number of release reports from exempt entities increased from 4 reports in 2006 to 148 reports in 2010 (Figure 1). After 2007, there was a rapid rise in the number of reports received from exempt entities each year, but unlike reports received from registered entities, the increase in report submissions did not subside in 2009 and 2010. The number of release reports submitted by exempt entities exceeded those submitted by registered entities in both 2009 and 2010. There was only one loss report submitted by an exempt entity during the entire time period. The submission rates per entity or per worker for exempt entities could not be determined because the total number of exempt entities or the number of workers at these entities is not known.

Ninety-two percent of the loss reports from regulated and exempt entities were associated with laboratory biocontainment (i.e., BSL) facilities instead of animal biocontainment (i.e., ABSL) facilities (Table 2). Most (~68%) of these events involved BSL-3 laboratories at registered facilities. Seven loss reports were submitted by registered entities performing animal work in ABSL-3 laboratories. Nearly two-thirds (65.9%) of all loss reports were inventory discrepancies. Other losses were categorized as discarded samples (14%) and shipping/transport errors (18%). There was only one loss report submitted by an exempt entity.

Eighty-five percent (537 of 639) of all release reports occurred in BSL facilities compared with ABSL facilities. However, more release reports were received from exempt than registered BSL facilities (290 vs. 247 reports, respec-

**Figure 3**

Annual rates of BSAT reports of loss and release events per 1000 workers with access approval.

The rate of release event reports and loss reports between 2004 and 2010 per 1000 workers with security clearances is graphically illustrated using lines connecting solid squares. The annual rate of release event reports release reports submitted to CDC per 1000 workers is indicated using open circles. The scale for both loss and release report rates are shown on the left side of the graph. The number of workers with approved security risk assessments each year that was used to calculate the rates shown in this graph is also indicated for each year using “X” symbols. The reference scale for the number of approved workers is shown on the right side of the graph. The numerical values for loss and release rates as well as the number of approved workers are shown above each symbol.
Ninety-one percent of reports from exempt laboratory BSL facilities involved BSL-2 laboratories, whereas most (~68%) of these reports from registered entities were associated with BSL-3 facilities. Most (98 of 102) of the reports from ABSL facilities were submitted by registered entities. Approximately 90% of these reports from registered entities involved ABSL-3 containment facilities. There were three reports from exempt ABSL-2 facilities and 1 from an exempt ABSL-3 entity (Table 2).

The number and types of BSATs described in the release reports could be determined for both registered and exempt entities. The total number of each type of agent is shown in Table 3 along with information about the numbers of agents reported by registered and exempt entities. There were 22 release reports in which more than one BSAT was involved. The total number of agents reported in the 639 release reports received by CDC was 676. Both registered and exempt entities listed bacterial species as the most predominant type of agent in these reports. However, bacteria constituted a substantially larger fraction of all agents reported by exempt entities compared to registered facilities (81% vs. 63%, respectively). Sixteen percent of agents reported by exempt entities were fungi but this type of agent was rarely reported (<3% of total agents) by registered facilities. Together, bacterial and fungal agents accounted for 97% of all agents reported by exempt entities. Viral agents were very rarely (<2% of total agents) reported by exempt entities but accounted for 23% of all agents associated with release reports submitted by registered entities. Release reports involving rickettsial agents were uncommon overall (3.6% of total) and extremely rare at exempt entities (0.7% of exempt total). There was only 1 report from an exempt entity that involved a toxin whereas 22 reports involving toxins were submitted by registered entities.

The annual distribution, type of select agent, type of entity and type of containment facility in which the 11 laboratory acquired infections occurred that were reported to CDC between 2004-2010 are shown in Table 4. There were five LAIs in 2004, two cases in 2007, one case in both 2008 and 2009, and two cases reported in 2010. There was only 1 TLR incident in which more than one LAI resulted from the release. This release occurred in 2004 and resulted in lab-acquired tularemia in three lab workers. Ten of the 11 LAIs involved bacterial agents whereas the remaining case resulted from exposure to a fungal agent.

LAIs occurred in both registered (8 cases) and exempt (3 cases) facilities. The majority of reports (5; some reports involved more than one case) and cases (7) involved BSL-2 laboratories in both registered and exempt entities. The remaining 4 LAIs occurred in BSL-3 laboratories located in registered facilities. All LAIs reported by exempt facilities occurred in BSL-2 laboratories.

There were 3412 approved shipments of BSAT between 2004 and 2010. There was only 1 shipment that was lost during transit. An investigation by law enforcement agencies concluded that the shipment was destroyed during shipment.

**Discussion**

This is, to the best of our knowledge, the first formal report describing the results of a national BSAT TLR reporting system focusing on high risk biological agents. After monitoring over 300 registered entities, that possess, use, or transfer BSATs each year between 2004-2010, the key findings from the present study include: 1) there were no reports of a theft of any BSAT between 2004-2010; 2) there was only one instance out of more than 3400 shipments in which a select agent was actually lost during ship-
ment; 3) there were only 11 laboratory acquired infections (LAIs) reported to CDC during the reporting period (1.2 per 10000 registered workers; 4) there were no fatalities that resulted from any of these infections; and 5) there were no reported cases of secondary transmission to other humans.

One of the most serious dangers to public health and safety addressed by the select agent regulations is the threat posed by the intentional misuse of BSATs as weapons of mass destruction against the United States population and the economic interests of the nation. In response to these concerns, a number of security requirements have been implemented in laboratories and are routinely reviewed by CDC to prevent the theft, loss or release of BSATs including physical security enhancements of facilities registered to possess BSATs, performance of security risk assessments for workers with access to BSATs, and requirements for improved accountability for these materials.

One of the key provisions for improving accountability of BSATs in the United States was the establishment of the regulatory requirement that all entities in possession of BSATs must keep accurate and current inventory records of BSATs held in long term storage (42 CFR § 73.17). Prior to the publication of the current select agent regulations in 2002, there were no regulatory requirements for maintaining any inventory records of BSATs present in laboratories. Although most laboratories in possession of BSATs did have inventory records before promulgation of the inventory record keeping requirement, there was considerable variation in the accuracy and completeness of these records. For many entities with large collections of BSATs, including those with archival materials, generation of accurate inventory records for all BSATs in their possession required substantial resources and time to complete. In order to ensure and maintain ongoing compliance with these inventory accountability requirements, CDC routinely con-

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**Table 3**

Number and type of BSATs associated with release reports received by CDC.

<table>
<thead>
<tr>
<th>Agent Type</th>
<th>All Entities</th>
<th>Registered Entities</th>
<th>Exempt Entities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># (%)</td>
<td># (%)</td>
<td># (%)</td>
</tr>
<tr>
<td>Toxins</td>
<td>23 (3.4)</td>
<td>22 (5.8)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>Fungi</td>
<td>58 (8.6)</td>
<td>11 (2.9)</td>
<td>47 (15.9)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>479 (70.9)</td>
<td>238 (62.6)</td>
<td>241 (81.4)</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>24 (3.6)</td>
<td>22 (5.7)</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Viruses</td>
<td>92 (13.6)</td>
<td>87 (22.8)</td>
<td>5 (1.7)</td>
</tr>
<tr>
<td>Total</td>
<td>676 (~100)</td>
<td>380 (~100)</td>
<td>296 (100)</td>
</tr>
</tbody>
</table>

* = percent of total reports in category for 2004-2010

---

**Table 4**

Laboratory Acquired Infections caused by BSATs between 2004-2010.

The annual distribution, type of select agent, type of entity and type of containment facility in which the 11 laboratory acquired infections occurred that were reported to CDC between 2004 -2010.

<table>
<thead>
<tr>
<th>Year</th>
<th>Agent</th>
<th># Cases</th>
<th>Entity type</th>
<th>Laboratory Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td><em>Brucella melitensis</em></td>
<td>1</td>
<td>Registered</td>
<td>BSL 2</td>
</tr>
<tr>
<td>2004</td>
<td>Coccidioides species</td>
<td>1</td>
<td>Registered</td>
<td>BSL 3</td>
</tr>
<tr>
<td>2004</td>
<td>Francisella tularensis</td>
<td>3</td>
<td>Registered</td>
<td>BSL 2</td>
</tr>
<tr>
<td>2007</td>
<td>Brucella melitensis</td>
<td>1</td>
<td>Registered</td>
<td>BSL 3</td>
</tr>
<tr>
<td>2007</td>
<td><em>Brucella melitensis</em></td>
<td>1</td>
<td>Exempt</td>
<td>BSL 2</td>
</tr>
<tr>
<td>2008</td>
<td>Brucella melitensis</td>
<td>1</td>
<td>Registered</td>
<td>BSL 3</td>
</tr>
<tr>
<td>2009</td>
<td>Francisella tularensis</td>
<td>1</td>
<td>Registered</td>
<td>BSL 3</td>
</tr>
<tr>
<td>2010</td>
<td><em>Brucella suis</em></td>
<td>1</td>
<td>Exempt</td>
<td>BSL 2</td>
</tr>
<tr>
<td>2010</td>
<td><em>Brucella suis</em></td>
<td>1</td>
<td>Exempt</td>
<td>BSL 2</td>
</tr>
</tbody>
</table>
ducts inventory “spot” checks of BSAT inventories during inspections. These spot checks have provided a mechanism through which the accuracy of BSAT inventories can be monitored.

Another important component of the inventory accounting responsibility is the requirement that all entities in possession of BSATs must immediately report any theft and the loss, even temporarily, of these materials (42 CFR § 73.19[a]). This immediate reporting requirement allows a response by law enforcement and regulatory agencies to be initiated in a timely manner. After these discrepancies are identified and reported to CDC or APHIS, a review by CDC of the circumstances surrounding this event is conducted that includes notification of the FBI in cases where the discrepancy cannot be rapidly reconciled.

The shipment of BSATs also represents an activity that could result in the theft or loss of these materials. Accordingly, the select agent regulations require pre-shipment authorization by either CDC or APHIS and notification to the regulatory agencies when materials leave the shipper and arrive at their destination. There were 3412 successful shipments of BSATs approved by CDC between 2004 and 2010. There was only one shipment that was lost during shipment between registered entities. It was determined by law enforcement officials that this shipment was destroyed during transit.

The select agent regulations also address the threat to public health and safety posed by an accidental release of BSATs from facilities using these materials for scientific or commercial purposes. Regulated entities “must develop and implement a written biosafety plan that is commensurate with the risk of the agent or toxin, given its intended use.” (42 CFR § 73.12[a]). There is also the additional requirement, that “the biosafety and containment procedures must be sufficient to contain the select agent and toxin.” (42 CFR § 73.12[b]). In the event that the biosafety and containment procedures in place do not contain the BSATs, the select agent regulations require that all entities that possess, use, or transfer BSATs must immediately report any release to CDC or APHIS. Prior to the implementation of the provisions of 42 CFR Part 73, there was no systematic reporting system at any level of government that monitored the number of laboratory workers and the type of infections and exposures associated with this occupation (Sewell, 1995).

Biological laboratories, and the work done therein, can be complex and the determination that a a release from primary containment has occurred may not be a straightforward process. Accordingly, the Federal Select Agent Program has provided the regulated community with guidance, including example scenarios, on the types of situations that would be considered to be releases and therefore reportable. This guidance can be found at the National Select Agent Registry web site (www.selectagents.gov). We have observed a significant increase in TLR Reports submitted to CDC since this guidance was first posted in early 2008. One possible explanation for this increase could be the outreach and education efforts that DSAT has conducted on the TLR reporting requirements. These efforts include a guidance document that was published in January of 2008 as well as more thorough inspections. The requirement for reporting all TLR events immediately after the occurrence or recognition of the event enables CDC to identify and assess any potential public health or security threats posed by the event. In addition, any time sensitive steps needed to protect workers from potential risks resulting from the release of a BSAT are identified as soon as possible after notification. CDC also uses information provided in the TLR Reports, as well as information collected through other sources such as verbal communications, written correspondence during the post-event evaluation process, information obtained during inspections and investigations of the reported event to identify new approaches for improvements in safety and security practices. Immediate reporting of exposures to BSATs that may pose serious public health threats also allows a more effective provision of assistance to entities that lack experience with medical or epidemiological issues associated with these agents, and provides occupational health professionals the opportunity to intervene with medical surveillance and countermeasures earlier in the potential infection process, when these measures are likely to be more effective. Ironically, intervening with prophylactic therapies early in the response to the report may obscure the number of actual infections resulting from release events of BSATs.

Other studies have attempted to assess the risk of infection associated with work in biomedical laboratories (Wedum & Kruse, 1969; Miller et al., 1987), but have encountered difficulties in achieving this goal due to problems with identifying subclinical infections, atypical incubation periods and routes of infection (Sewell, 1995). Other factors such as fear of reprisal or embarrassment may contribute to underreporting of laboratory acquired infections.

The specificity of these reporting criteria should be considered when reviewing the data presented in this manuscript. These reported events do not describe situations where a total loss of containment of the BSATs and a release into the environment outside of the containment facility may have occurred. Since there are redundant containment barriers and procedures in place in all registered facilities that work with BSATs in the United States, these additional safety barriers and procedures greatly reduce the likelihood that any BSAT will escape outside of the laboratories. Any risk for a release of a BSAT beyond the redundant containment barriers of the facility is immediately assessed based on the information we receive during the initial and follow up reports.

Overt occupational exposures represent a recognizable event in which the worker exposed to the BSAT may be at risk for developing a laboratory acquired infection. This type of release can be minimized through training, proper use of personal protective equipment (PPE) and improved engineering controls for sharp instruments (e.g., contaminated needles). None of the LAIs reported to CDC resulted
from overt exposures to BSATs (Table 4).

The second type of release event defined in the regulations (i.e., a release outside of the primary containment barriers); however, can represent a less recognizable threat to workers in biocontainment laboratories. All 11 LAIs resulted from either unrecognized and/or unreported exposures, presumably through the aerosol release of the BSATs. These observations are entirely consistent with studies by Pike (1976) who found no distinguishable accidents or exposure events in more than 80% of LAIs. Harding and Byers (2000) also reported only a small number of recognized containment breaks in a study of LAIs.

The regulatory requirement or pre-access approval by CDC for all individuals working with BSATs in CDC-registered facilities allows for a reasonable estimate of the population for which the incidence of LAIs can be determined. During the seven years in which report data was available for this study, the average annual rate of LAIs in BSAT facilities registered with CDC was 1.6 per 10,000 authorized workers. In order to place this rate in an overall context of occupational illnesses in similar workplaces, a comparison may be made with incidence rates of occupational illnesses in scientific research and development facilities, as reported by the Department of Labor (NAICS Category 5417, rates from 2006 through 2010). These rates ranged from a high of 22.3 illnesses per 10,000 workers in 2006 to a low of 15.9 illnesses per 10,000 workers in 2009. Although these metrics may not be directly comparable, this information suggests that BSAT facilities are at least as safe, if not safer, than facilities in the overall U.S. research and development sector.

The reporting requirement of any suspected TLR provides information about the characteristics of events occurring in BSAT laboratories and the circumstances surrounding these events. Initial analysis of this information has provided some insights that may improve biosafety practices in laboratories working with BSATs under differing conditions. For instance, detection of differences between registered entities and entities exempted from registration requirements, such as clinical and diagnostic laboratories, has allowed CDC to consider targeted recommendations that more appropriately address the risks associated with different laboratory operating conditions in these types of facilities. The predominance of potential release events at exempt entities involving just two types of agents (i.e., bacteria and fungi), which are predominantly manipulated in BSL-2 facilities, contrasts with the broader spectrum of agents reported by registered entities that occurred most frequently in BSL-3 laboratories. The marked differences between these entity types in the frequency of potential release events with viral agents and toxins were also noteworthy. The requirement for exempt entities to report the identification of BSATs and any TLR events provides a mechanism for agencies responsible for the regulatory oversight of BSATs to obtain assurance that these materials are either destroyed or transferred to facilities with approved security measures in place. These reports also allow assessment of the factors that are associated with potential release events in animal biosafety facilities and maximum containment laboratories.

A recent report by the Trans-Federal Task Force on Optimizing Biosafety and Biocontainment Oversight recommended the establishment of a non-punitive incident reporting system for high- and maximum containment biological laboratories that possess, use, or transfer both BSAT and non-BSAT agents. Such a system, the Task Force reasoned, could provide valuable information in assessing risk and developing more effective biosafety programs in these facilities. Although the CDC TLR reporting system is a regulatory requirement and failures to report could potentially result in punitive actions by the federal government, our experience suggests that the system envisioned by the Trans-Federal Task Force could provide valuable insights related to incidents in a wide range of biological laboratories. The data provided through this system could be helpful in identifying specific combinations of agents and/or activities for which risk assessment and control activities can be focused. This expansion of monitoring to non-BSAT associated events may provide a broader understanding of the incidence of such events and inform biosafety and biosecurity guidance.

The findings in this report are subject to a number of limitations. The number of reports received by CDC likely is an underestimate of the true number of suspected losses and releases of BSAT, especially in the early years of the program because there was little encouragement for exempt entities to report TLR events prior to 2005 and guidance regarding TLR reporting was not provided to the regulated community until 2008.

Additionally, releases are likely to have been unrecognized and therefore unreported. In spite of these limitations, the reports that have been submitted contain a substantial amount of information about potential problems with the safety and security of BSATs in the United States. This information can provide insights into approaches to improve safety and security in these facilities.

The data collected by CDC between 2004 and 2010 of monitoring suspected BSAT TLRs indicate that the risk of exposure to BSATs managed by US laboratories to the general population is low. However, the potential misuse of these agents and toxins remains a risk to public health at the national and international level. Effective biosafety and biosecurity programs in US laboratories will continue to be critical tools to effectively manage this risk.

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addition, the assistance of Dr. Sarah Crawford for advice with statistical analysis is also appreciated. Finally, Mrs. Torrie D’Avilar’s assistance with the processing of information from the reports of release events submission should also be recognized. *Correspondence should be addressed to Richard Henkel at bzz3@cdc.gov.

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Trans-Federal Task Force on Optimizing Biosafety and Biocontainment Oversight. Available at: www.phe.gov/Preparedness/legal/boards/biosafetytaskforce/Pages/default.aspx

Training Announcement—6th Annual Leadership Institute

The 6th Annual Leadership Institute “Managing Unmanageable Situations: Management Techniques and Strategies for Biosafety Professionals” will be held from April 15-18, 2013 at the Charleston Marriott in Charleston, South Carolina.

The core focus of the 2013 ABSA/Emory/ERGRF Biosafety Leadership Institute will be to provide management instruction, guidance, and practice for biosafety professionals to utilize in designing and implementing effective biosafety programs in their home institutions.

Each participant of the 2013 Leadership Institute will leave with a personalized plan that complements the missions and goals of their own respective institutions. By engaging with an expert faculty and networking with peers, each participant will be equipped to demonstrate how their program plays a vital role in facilitating research and enhancing the sustainability of their respective institutions. For more information, please visit www.absa.org/eduleadership.html.
Administering Needs-assessed Bioterrorism Curricula to Public Health Professionals Using Active-learning Strategies

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Abstract

Acts of bioterrorism, such as the “anthrax letters” in 2001, exposed public health professionals to potentially high concentrations of unknown biological organisms. After an event such as the “anthrax letters,” it became apparent that public health professionals require continual training and knowledge about proper biological safety level 3 (BSL-3) practices and procedures. To assess specific content areas where training was needed, public health professionals in northeast Ohio were given an original assessment. Prior needs assessments had warranted the development of three 2-day courses based on BSL-3 practices and government regulations. These courses stimulated learning by combining open-ended questioning and hands-on exercises to promote group discussion. Testing instruments were administered before and after the courses and analyzed by paired t-test to assess improvement in participant knowledge. Five questions from this test were used as content measures to assess learning in specific content areas. Learner attitudes were assessed following course completion via survey data. Participants averaged 67.7% ± 9.2 on testing instruments prior to courses as compared to 86.0% ± 8.6, following course completion (p < 0.001). Improvement was significant (p < 0.05) in all content areas measured. Eighty-two percent of participants responded that they would change their practices based on information learned from the course. These data coupled with learner attitudes demonstrate a significant increase in participant knowledge among public health professionals and a willingness to change existing practices through the integration of new concepts.

Keywords
Biosafety, laboratorians, active-learning, continuing education, and bioterrorism

Introduction

Incidents of bioterrorism worldwide over the past 2 decades have propelled people into varying states of awareness and fear (Klietmann & Ruoff, 2001). The 2001 anthrax letters alone impacted 5 states and 17 cities, and resulted in the death of at least five people, elevating bioterrorism vigilance to chronic levels (Jernigan et al., 2001; Jernigan et al., 2002; Steinbruner & Harris, 2003). While high quantities of anthrax and other select agents are typically limited to the confines of high-security biosafety level 3 (BSL-3) laboratories, the anthrax attacks on the U.S. postal system changed this. During this time of chaos, “white powders” suspected to be anthrax were shuttled through hospitals and health departments where personnel were ill-prepared to handle them (Snyder, 2003).

The vulnerability of the United States to bioterrorist attacks led to increased government spending for and oversight of select agent research and containment facilities (Atlas, 2005; Schwellenbach, 2005). The National Institutes of Health’s (NIH) biodefense research funding alone increased from $25 million in 2001 to $1.7 billion in 2011 (Atlas, 2005; Kaiser, 2011), funding an increasing number of investigators working with select agents (Schwellenbach, 2005). Increased funding spurred recommendations from scientific organizations to: 1) increase laboratory biosecurity; and 2) increase laboratory safety and better train laboratory personnel to prevent accidental releases and laboratory-acquired infections in the event of natural outbreaks or acts of terrorism. The American Society for Microbiology (ASM) made recommendations to the Energy and Commerce Subcommittee on Oversight to: 1) require mandatory NIH and Centers for Disease Control and Prevention (CDC) training for individuals involved with BSL-3 and BSL-4 laboratories; 2) form NIH Regional Centers for Excellence serving roles in biosafety training and information; 3) follow strict adherence to NIH regulations and guidelines set forth in Biosafety in Microbiological and Biomedical Laboratories (U.S. Department of Health and Human Services, 2009); and 4) provide better surveillance and reporting of laboratory-acquired infections (ASM, 2007).

Legislation more stringently regulating access and use of select agents quickly ensued (Marmagas et al., 2003). Among this new legislation were policies enacted through the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (U.S. House of Representatives, 2002) requiring investigators possessing, transporting, or using agents or toxins (deemed a threat to public, animal, or plant health) to notify the CDC or the Department of Agriculture’s Animal Plant Health Inspection Service (APHIS) to increase the monitoring of select agent use. Additionally, Senate Bill 485 (U.S. Senate, 2009), known as the Select Agent Program and Biosafety Improvement Act of 2009, proposed requiring all users of BSL-3 laboratories to have extensive, mandated training. However, mandated training of select agent users has been slow to follow as S. 485 was never passed into law.

As a consequence of this hyper-vigilance and the increased number of personnel working with select agents,
the almost six-fold increase in federal funding for bioterrorism preparedness included funds for training laboratory personnel (Baker Jr et al., 2005; Berger et al., 2009; McHugh et al., 2004). One avenue for increased preparedness education and training came through CDC-funded Centers for Public Health Preparedness, originally developed in 2000 and reorganized as the Preparedness and Emergency Response Learning Centers (PERLC) in August 2010 (CDC, 2012; Fink, 2003).

The vast amount of funding dedicated to and the abundance of recommendations directed toward enhanced safety and security within BSL-3 laboratories resulted in an abundance of content-heavy curricula released via the internet. Although some laboratory personnel sought and completed trainings based on this curricula, the continued reporting of laboratory-acquired (Harding & Byers, 2006) and research-acquired (Kaiser, 2007; Lawler, 2005) infections suggests that limited numbers of training opportunities are available or that a disconnect exists when translating the theoretical constructs of lab safety into best practices that are incorporated into the workplace. In a changing work environment with the potential threat of select agent exposure, public health professionals must continually train throughout their careers to assimilate new information to remain competent, proficient, and safe in the workplace (Fiester et al., 2010).

Curricula for laboratory professionals should develop critical thinking skills in adult learners, allowing them to adapt in a rapidly changing workplace (Kiltz, 2009). One way to promote this critical thinking in laboratory workers is to employ an active learning environment (Paul & Binker, 1990; Severiens & Schmidt, 2009), similar to strategies successfully used in the undergraduate science classroom, that supports public health professionals in obtaining “up-to-date” content in a way that encourages them to integrate the best practices into their workplace. The authors have previously demonstrated the use of active learning and peer education as effective teaching and learning methods for hospital laboratorians (Fiester et al., 2010; Paul & Binker, 1990). Peer education gives students the opportunity to share experiences and knowledge with one another, thus providing real-life examples of course material. While peer education was the primary teaching style used to deliver content in this study, a facilitator is still needed to maintain the continuity of course material and to ensure all content is delivered effectively. The facilitator guides, monitors, and directs peer interactions by selecting the material for group discussions and facilitating the course of these discussions (Damon, 1984).

Needs assessments were utilized in this study to establish the need for bioterrorism preparedness training among public health professionals. A need for training was received from these data, resulting in 2-day bioterrorism preparedness courses being designed. To test the efficacy of using active-learning strategies to teach bioterrorism curricula, a course was created in which hands-on activities, case studies, and personal experiences were utilized to create an active-learning environment for adult learning. Pre-tests, post-tests, and post-course evaluations were performed to assess learning and gauge learner attitudes, respectively.

Methods

Needs Assessment Survey

One hundred and eight public health laboratory workers and 15 public health administrators were surveyed to evaluate: 1) confidence in performing tasks associated with biological agents and BSL-3 practices and procedures; 2) need for training associated with biological agents and BSL-3 practices and procedures; 3) likelihood of participation in courses; 4) previous training; 5) training preference; 6) requirement for continuing education credit; 7) timing restrictions; and 8) other demographics specific to survey participants. Survey questions were in either a binary (yes/no) format or coded to a Likert scale. Survey questions evaluating confidence performing tasks and training needs were coded to a five-point Likert scale: highly confident or highest priority for training (value = 1); confident or high priority for training (value = 2); somewhat confident or somewhat of a priority for training (value = 3); not very confident or not a very high priority for training (value = 4); or have not addressed/not at all confident or no need at all for training (value = 5). The Likert values obtained for administrators and laboratory workers were averaged and reported as the mean ± standard deviation. A priority for training was perceived if a mean score of 3 or less was obtained from training priority polling data. The confidence of surveyed public health professionals to complete tasks was considered weak if a mean value of 3 or more was obtained from confidence polling data. Both administrators and laboratory workers were also polled to ascertain the degree to which they felt prepared for a bioterrorist event. Possible responses were prepared at an “advanced” level, “intermediate” level, “awareness” level, or not prepared.

Curriculum Development and Delivery

BSL-3 curricula were developed based on training needs determined by survey data and recommendations and guidelines from the CDC, NIH, ASM, and Ohio Department of Health. Three courses were held that consisted of group activities, peer-to-peer teaching, and hands-on components. PowerPoint (Microsoft Corporation, Redmond, WA) content slides were developed as reference material and used to direct open-ended discussions, facilitating problem-based and scenario-based learning. As a scenario-based learning strategy, students were instructed to conduct experiments with an “unknown” sample (Glo Germ™ powder, Moab, Utah) using proper practices and procedures. These experiments involved proper procedures for donning and doffing personal protective equipment (PPE) and using and maintaining biological safety cabinets (BSCs). Another scenario was introduced in which the “unknown” sample was spilled, allowing students to practice decontamination strategies. Molecular microbiological techniques for pathogen identification (rt-PCR) and toxin quantification were
also included in the practice-based curriculum. As an adjunct to these exercises, interrupted case studies of lab risks and mitigation strategies were utilized. Students were allowed to work with peers to perform experiments, as they would in their own laboratories. These exercises were videotaped, and footage was edited, made anonymous, and shared with students. Videotaping allowed facilitators to cite specific areas where proper practices and procedures were not followed, thereby tailoring course content to the students’ needs. Following facilitator-based instruction, students repeated the experiments, allowing them to integrate new knowledge and concepts. The combination of open-ended questioning, peer-to-peer teaching, group exercises, and hands-on activities was specifically designed to create an active-learning environment for adult learners in which transformative learning would elicit critical thinking in the workplace (Marbach-Ad et al., 2009; Severiens & Schmidt, 2009).

Curriculum Efficacy
A multiple-choice testing instrument was designed and administered to participants before and after the courses. This assessment tool was used to gauge knowledge both before and after training. By comparing scores of the identical pre-test and post-test using a paired t-test, the efficacy of the course could be analyzed. Selected questions focusing on select agents, N-95 masks, secondary barriers, HEPA filters, and glove use were used as content measures and individually analyzed to assess learning in these areas of the curricula. Examples of these content measure questions were: 1) “Which of the following is true regarding the N-95 mask?” a. protects as well as a surgical mask; b. should be replaced after each use; c. supplies oxygen; d. reduces allergens and pathogens with a 95% efficiency; or e. filters out chemicals; and 2) “HEPA filters exclude…” a. gases and vapors only; b. particles only; c. gases, vapors, and particles; d. air; or e. none of the above. Statistical analyses were conducted using GraphPad InStat (GraphPad Software, Inc., San Diego, CA). Significance was set \( \alpha \) priori at \( p \leq 0.05 \).

Learner Attitudes
Participants completed the evaluations after the courses. Questions on the evaluations were multiple-choice in nature and coded to a five-descriptor (strongly agree = 5, to strongly disagree = 1) Likert scale. These questions were used to measure learner attitudes pertaining to certain aspects of the training. Participants were asked if they were in agreement with the following statements: 1) instructional materials were beneficial to learning; 2) the hands-on portion met training needs; and 3) I will change my practices and procedures after participation in this course. Participants were considered to be in agreement with a statement if the geometric mean of responses was greater than or equal to three. Analysis of the evaluations was conducted using GraphPad InStat (GraphPad Software, Inc., San Diego, CA).

Results

Needs Assessment Survey
Data obtained from the needs assessment survey were used to develop course content, structure, and delivery. The majority of polled administrators felt that they were prepared only at the “awareness” level or not prepared at all for a bioterrorist attack, with 40% responding preparedness at the “awareness” level and 13.3% responding a complete lack of preparedness. Laboratory workers also felt unprepared, with 30.8% responding preparedness at the “awareness” level only and 44.9% responding a lack of preparedness. The majority of laboratory workers did not express confidence in any of the areas polled (Table 1). The lack of confidence is complimented with the majority of administrators responding a priority for training in all areas polled (Table 2). Laboratory workers felt training was needed for the detection of biological agents and weapons, laboratory readiness preparing for bioterrorism, and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (Table 2). While 80% of administrators had previous education or training that addressed issues related to bioterrorist threats, only 26.4% of laboratory

<table>
<thead>
<tr>
<th>Area of Bioterrorism</th>
<th>Mean Administrator Response ± Standard Deviation</th>
<th>Mean Laboratory Worker Response ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detecting biological agents and weapons</td>
<td>3.3 ± 1.0</td>
<td>4.1 ± 1.1</td>
</tr>
<tr>
<td>Detection and diagnosis to rule out select agents</td>
<td>2.9 ± 1.2</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>BSL-3 laboratory procedures</td>
<td>3.0 ± 1.5</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Laboratory readiness (preparing for bioterrorism)</td>
<td>2.9 ± 1.1</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>Public Health Security and Bioterrorism Preparedness and Response Act of 2002</td>
<td>3.5 ± 1.6</td>
<td>4.5 ± 1.0</td>
</tr>
</tbody>
</table>

*Scale ranges from highly confident (value = 1) to not at all confident (value = 5).
workers had similar training. When polled on their likelihood of participating in trainings conducted using traditional classroom instruction, less than half (41.1%) of the laboratory workers answered they would be likely to participate.

Curricula Efficacy

Student knowledge of BSL-3 concepts improved, as evidenced by post-test scores. Scores increased significantly from 67.7 ± 9.2% on pre-tests to 86.0 ± 8.6% on post-tests (p < 0.001) (Table 3). All measured content areas significantly improved between pre-tests and post-tests (Table 3). Most notable were improvements in knowledge of containment, including secondary barriers and HEPA filtration. The number of students responding correctly to the secondary barrier and the HEPA filtration content measures increased from 28% to 88% and 48% to 80%, respectively (Table 3). While improvement in knowledge of glove use was not as striking, significant improvement did occur. Eighty-eight percent of students responded correctly to the glove use content measure on pre-tests and 100% of students retained this knowledge on post-tests (Table 3).

Learner Attitudes

Post-course evaluations were administered to students to assess the efficacy of curricula structure. Questions were designed to assess behavioral change. Possible responses for each question ranged from strongly agree (value = 5) to strongly disagree (value = 1). Participants agreed with the statements, “the BSL-3 laboratory exercises were appropriate and met my training needs,” and “the provided instructional materials were beneficial to my learning,” answering with mean responses of 4.73 ± 0.45 and 4.92 ± 0.28, respectively (Figure 1). More importantly, students agreed they would “change their laboratory practices and procedures based on what they learned in the course,” replying with a mean response of 4.45 ± 0.82 (Figure 1).

Discussion

While the majority of administrators polled in this report had education or training that addressed issues relating to bioterrorist threats, only a fraction of laboratory workers had similar training. This is troublesome considering laboratory workers are at a greater risk of being in contact with harmful agents. A training deficit among public health professionals is supported further by pre-test data obtained during this study and the lack of confidence expressed by both administrators and laboratory workers on the needs assessment survey. Specifically, there was a lack of knowledge of secondary containment and HEPA filtration in 72% and 52% of participants, respectively.

Ongoing assessment of preparedness suggests that the United States and other countries are far from mounting successful responses to events of bioterrorism. The American Association for the Advancement of Science (AAAS), ASM, and individual researchers have cited the need for training the public health community (Berger et al., 2009; Fiester et al., 2010; Kiltz, 2009). Compounding the lack of preparedness among public health professionals is the lack of a standardized accreditation process for BSL-3 workers. No standards or accreditation currently exist for BSL-3 professionals; however, 70% is the standard required of BSL-2 medical technologists on the American Medical Technologists (AMT) examination. The Bioterrorism Training and Curriculum Development program (created in the Public Health Security and Bioterrorism Preparedness and Response Act of 2002) addressed the lack of standardization and accreditation, making recommendations for uniform training standards and certifications. These recommendations led to the inclusion of requirements for standardization and accreditation in the Weapons of Mass Destruction (WMD) Prevention and Preparedness Act of 2009 (Berger et al., 2009). The WMD Prevention and Preparedness Act of 2009 called for: 1) training standards; 2) risk assessment standards; 3) security standards; and 4) accreditation of training programs by the Secretary of Health and Human Services.

Whether exposure originates from an unintentional release of a select agent or, more likely, from a natural disease outbreak such as pandemic influenza, deficits in training must be addressed through continual training (Hartwig et al., 2009; Schwellenbach, 2005; Sewell, 2006; Weisfuse,
Based on survey data outlining the public health professionals’ disinterest in traditional classroom instruction, the education literature, and the effectiveness demonstrated in this study, training using active-learning teaching strategies appears a plausible avenue to achieve increased preparedness amongst public health professionals. AAAS cites active teaching methods as the most successful method for educating public health workers (Berger et al., 2009).

The aforementioned select agent preparedness course effectively utilized these active-learning techniques in the form of group activities, case studies, peer-to-peer teaching, hands-on components, and dynamic lectures directed by open-ended questioning to facilitate problem-based and scenario-based learning. Not only was significant improvement documented between overall pre-test and post-test scores (Table 3), but also significant improvement was demonstrated in knowledge within all measured content areas (Table 3). These data demonstrate that active-learning methods, such as the exercises involving manipulation of the “unknown” sample, allow students to collaboratively utilize critical thinking and affirm active-learning methods as an effective way to teach bioterrorism-related material.

An increase in participant knowledge post-course is not surprising considering that the high number of laboratory-acquired infections and the possibility of bioterrorism events spur adult learners’ interest and participation in

<table>
<thead>
<tr>
<th>Content Measure</th>
<th>% Students Meeting Content Measure</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre-test</td>
<td>Post-test</td>
</tr>
<tr>
<td>Select agents</td>
<td>76</td>
<td>92</td>
</tr>
<tr>
<td>N-95</td>
<td>68</td>
<td>96</td>
</tr>
<tr>
<td>Secondary barriers</td>
<td>28</td>
<td>88</td>
</tr>
<tr>
<td>HEPA filters</td>
<td>48</td>
<td>80</td>
</tr>
<tr>
<td>Glove use</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>Total Test Score Mean</td>
<td>66.4 ± 9.9</td>
<td>83.4 ± 8.3</td>
</tr>
</tbody>
</table>

Figure 1
Post-course evaluation of curricula structure. After completion of trainings, participants were asked if the instructional materials were beneficial, if exercises met training needs, and if they would change their practices based on knowledge gained from the trainings. Possible responses for each questions were strongly agree (SA, value = 5), agree (A, value = 4), neutral (N, value = 3), disagree (D, value = 2), or strongly disagree (SD, value = 1).
courses pertaining to select agents. Adult learners seem to prefer a learning atmosphere in which they can build off of one another’s experiences while incorporating new concepts. It is, therefore, not surprising that following active learning-based courses, 96% of participants scored greater than 70% on post-tests.

In addition to an overall improvement in test scores, participants responded with positive feedback to laboratory exercises and instructional materials (Figure 1). While the needs assessment demonstrated public health professionals’ lack of confidence in performing tasks, these participants also expressed that training was a priority. Once training was offered, knowledge of concepts associated with biological agents and BSL-3 practices and procedures increased significantly. In fact, the majority of students participating in the evaluation stated that they would change their laboratory practices and procedures based on the knowledge gained from the course. This implies that learners developed critical thinking skills that encouraged them to change their work practices to increase safety and security.

Bioterrorist attacks, increases in select agent research, as well as continual reports of laboratory- and research-acquired infections spur the public health professional to seek continual education to maintain competency and safety in the workplace. Survey and testing data led to the recommendation that continuing education be used to maintain workplace competency among public health professionals and that yearly needs assessments be completed to determine deficient areas for future course development. For future courses, the authors also recommend sending course participants an online testing instrument, consisting of questions equivalent to those used in the pre- and post-tests, 6 weeks after completion of the course. This instrument could more efficiently assess improvement in knowledge of the course material than the identical pre- and post-tests used in this study, where improvements in test scores could be partially attributed to familiarity with test questions. This follow-up test would also provide a way to gauge curricula retention, allowing for changes in curricular delivery and thus course improvement.

This study suggests training public health professionals using an active teaching model where case studies, group and hands-on activities, peer-to-peer teaching, and lectures directed by open-ended questioning are combined to ingrain creative problem-solving skills. This teaching style seems not only preferred by public health professionals, since the majority do not prefer traditional teaching styles, and effective as demonstrated by increases in participant knowledge following the course, but also transformative as the majority of participants intended to improve their laboratory practices and procedures based on the knowledge gained from the course. Data from this study demonstrate that an interactive teaching style not only increases knowledge of biological safety concepts among public health professionals, but also consequentially results in a changed workplace as workers integrate new concepts introduced in this educational setting.

Acknowledgments

This project was approved by the Kent State University Institutional Review Board for the study of human subjects. *Correspondence should be addressed to Shannon Helfinstine at shannon.helfinstine@aultmancollege.edu.

References


**Training Announcement—Principles & Practices of Biosafety (PPB)**

This is a comprehensive, interactive, 5-day course that introduces the essential elements of biosafety and provides extensive resource lists for use after the course. Interactive exercises are used throughout to provide hands-on experience and to encourage networking and problem solving among participants and instructors.

Upon completion of the course, participants will be able to: describe potentially hazardous biological materials, the risks associated with their use, and the means to minimize risk and to protect against or prevent release or exposure; discuss ways to provide effective technical expertise in situations involving potentially hazardous biological materials; and, identify, locate, and efficiently use key biosafety resources.

This course is designed for persons who are entering the profession and those with up to three years experience in biosafety. It is also suitable for persons who supervise biosafety professionals and for those who will benefit from additional knowledge of biosafety as a complement to their primary responsibilities.

To register for the PPB at the Embassy Suites Orlando—Lake Buena Vista South in Kissimmee, Florida from February 24 to March 1, 2013, visit www.absa.org/eduppb.html.
Washout Kinetics of Viral Vectors from Cultured Mammalian Cells

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1State Laboratory Basel-City, Basel, Switzerland and 2Safety Inspectorate, Liestal, Switzerland

Abstract

The handling of infectious viral vectors such as adenovirus type 5 (Ad5), lentivirus (HIV1), and vaccinia virus (VV) requires biosafety level 2 (BSL-2) containment. This also applies to modified vaccinia Ankara (MVA) and adeno-associated virus type 2 (AAV2) provided that the insert involves additional hazard. To transfer infected cells to the lower BSL-1 containment, viral particles have to be demonstrably cleared from the supernatant as specified by biosafety guidelines. With the objective to provide data about the washout kinetics using common culture techniques, the authors infected different adherent mammalian cells with the aforementioned viral vectors. The supernatant of the cell culture was subsequently monitored for the presence of viral nucleic acids during various steps of washing and cell passaging. Complete clearance could be demonstrated for Ad5 when infecting Hela cells and for lentivirus (HIV1) in HEK293T cells. No or an undefined clearance was detected for AAV2, VV, and MVA in Vero-B4 cells and Ad5 after infection of HEK293 cells. Results demonstrated that virus persistence in the supernatant was greatly influenced by the washing procedure, the number of passages, and the vector titer, as well as the type of host cell line. The authors therefore conclude that procedures for clearance cannot be predefined for given virus-host systems. An analysis of the supernatant should be performed for each individual experimental setup prior to downgrading the risk class and subsequently the containment level used.

Introduction

Adenovirus type 5 (Ad5), HIV1-derived lentivirus, adeno-associated virus (AAV), and vaccinia viruses are well-established tools for gene transfer in vitro and for gene therapy (Bouard et al., 2009; Howarth et al., 2010). These vectors exhibit distinct characteristics concerning transgene delivery and expression as well as replication capacity and biosafety. Ad5 and lentiviral (HIV1) vectors have been generated by removing parts of the original viral genome. For replication, they depend on specific host cells or the addition of helper plasmids (Palmer & Ng, 2005; Pauwels et al., 2009). Supranational guidelines for contained use of these viral vectors agree that they be handled at biosafety (containment) level 2 (BSL-2) (Eidgenössische Fachkommission für biologische Sicherheit, 2009; Health and Safety Executive (HSE), 2007; National Institute of Health (NIH), 2011; Zentrale Kommission für Biologische Sicherheit (ZKBS), 1997). Modified Vaccinia Ankara (MVA), which has been generated by serial passage in chicken embryo fibroblasts (CEF), exhibits a highly reduced replication capacity (Meyer et al., 1991). Thus, BSL-2 handling is compulsory only when the transgene implicates a supplementary risk (Eidgenössische Fachkommission für biologische Sicherheit, 2009; Health and Safety Executive (HSE), 2007; National Institute of Health (NIH), 2011; Zentrale Kommission für Biologische Sicherheit (ZKBS), 2002). Adeno-associated viruses (AAV) are generally regarded as non-pathogenic because replication and release of viral particles take place only during superinfection with a helper virus (adenovirus, herpes simplex virus [Berns & Giraud, 1996]). The most commonly used serotypes of AAV (AAV2, 3, 5) have been classified as risk (hazard) group 1 (RG1), unless helper viruses are employed (Eidgenössische Fachkommission für biologische Sicherheit, 2009; Health and Safety Executive (HSE), 2007; National Institute of Health (NIH), 2011; Zentrale Kommission für Biologische Sicherheit (ZKBS), 2005). However, evidence for insertional mutagenesis at the preferred integration site AAVS1 in the host genome, as well as the fact that cellular genotoxic stress can induce a lytic response, has led to considerations about a reassessment of AAV (Donsante et al., 2007; Lambert, 2008; Yalkinoslu et al., 1988).

By biosafety regulation, derogation of virally infected cells from RG2 to RG1, and thus their contained use at BSL-1 instead of BSL-2, is possible given that no viral particles are detectable in the supernatant of the infected cells. Since the user prefers to carry out experimental work post infection at BSL-1, the time and the procedure required to clear all viruses from the cell culture supernatant are desirably minimal. As an official biosafety laboratory, the authors support the federal authorities in Switzerland in issues related to the Swiss Ordinance on the Contained Use of Organisms (The Federal Authorities of the Swiss Confederation, 1999). They analyzed procedures for clearing viral particles from cell culture for individual strains of Ad5, lentivirus (HIV1), VV, MVA, and AAV2. Several types of adherent mammalian cells were infected with these viral vectors and the supernatant of the cell culture was monitored for the presence of viral nucleic acids after infection and subsequent cell passage. As a conclusion, a general statement of the derogation of the containment level is made.
Materials and Methods

Plasmids, Viral Specimens, and Cell Lines

Adenovirus Type 5 (Ad5) construct Ad5ΔE1GFP (E1 region of Ad5 replaced by a green fluorescent protein expression cassette; replication-deficient) (Ehrengruber et al., 2000; Qu et al., 1998) was a kind gift from Markus Ehrengruber, Kantonsschule Hohe Promenade, Department of Biology, Zürich, Switzerland. The pLL3.7 plasmid (lentiviral vector) (Rubinson et al., 2003) as well as the helper plasmids pMD2.G, pRSV-Rev, and pMDLg/pRRE (Dull et al., 1998) were obtained from Addgene Inc. (Cambridge, MA, USA). Lentivirus (HIV1) pLL3.7 stock solutions were prepared as described earlier (Bagutti et al., 2011) and concentrated by Lenti-X™ Concentrator (Clontech, Takara Bio Europe, St. Germain-en-Laye, France) according to the manufacturer’s protocol. Adenovirus associated virus serotype 2 (AAV2; ATCC-VR-680, [Hoggan et al., 1966]) was purchased from LGC Standards. HEK293 (ACC 305), HEK293T (ACC 635), Hela (ACC 57), and Vero-B4 (ACC 33) cells were obtained from DSMZ (Braunschweig, Germany).

Cell Culture

Cells were routinely cultivated in standard cell culture flasks (TPP, VWR, Dietikon, Switzerland) at 37°C with 6% CO2 using standard culture medium (Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Buchs, Switzerland) as well as either 27 μg/ml Genetin® (G418; Gibco, Invitrogen, Basel, Switzerland) for Vero-B4 or L-Glutamine (200 μM), Penicillin (10 units/ml), and Streptomycin (10 μg/ml) (Sigma) for HEK293, HEK293T, and Hela. Routine procedures were performed as follows: The cells were washed by adding and subsequently removing 1x PBS (Sigma); repeated once. Passaging of the cells was performed by washing (as described above) followed by the addition of 1x trypsin/EDTA (Sigma) for approximately 2 minutes before stopping the reaction and removing the cells by adding cell culture medium (in a five-fold excess to trypsin). The cells were resuspended directly in 1/5 to 1/20 dilutions depending on the cell density.

Viral Infections

Ad5 infection of HEK293 and Hela cells was performed by adding Ad5ΔE1GFP stock solution (two separate viral preparations) to wells of a 12-well cell culture plate (TPP, VWR, Dietikon, Switzerland) before the cells were added, leading to a confluency of 70% (Bagutti et al., 2011). Twenty-four hours later the medium was exchanged (standard protocol) and the cells were incubated for another 3 days. Where indicated, additional washing steps (twice with PBS at room temperature or twice with standard supplemented DMEM at 37°C for 5 minutes each) were carried out prior to medium exchange. The cells were then passaged routinely up to 14 days or until cell death occurred. For lentivirus (HIV1) infection of HEK293T, the cells were seeded in advance in poly-L-lysine-coated 6-well plates (BD Biocoat™ [Becton Dickinson, Franklin Lakes, NJ], VWR, Switzerland) to reach a confluency of 70% the day of infection. One hour prior to the addition of pLL3.7 lentiviral stock solution (two separate viral preparations), the cells were treated with 8 μg/ml Polybrene. Five hours post infection, the cells were washed twice rigorously with 1x PBS and incubated for another 3 days. The cells were then passaged routinely as indicated for up to 15 days. For AAV2 infection, HEK293 and Hela cells were seeded the previous day in 6-well plates to reach a confluency of 70% on the day of infection. The medium was removed except for 500 μl before 10 μl of AAV2 stock solution (ca. 104 and 106 genome copies/ml) were added and incubated in the presence or absence of 3 μM etoposide (Sigma-Aldrich, Buchs, Switzerland). After 2 hours, the medium was replenished to 3 ml. The cells were then passaged routinely as indicated until cell death occurred or for up to 14 days. VVeGFP and MVA infection of Vero-B4 was performed by adding VVeGFP or MVA, respectively, and basically according to the same protocol as for AAV2 (with the deviation that the medium did not contain any etoposide and the medium was replenished 1 hour post infection to 1.5 ml). The cells were passaged routinely as indicated for up to 8 days and 35 days, respectively, or until cell death occurred.

Sampling and DNA/RNA Extraction

To extract nucleic acids, 200 μl of supernatant or 200 μl cell suspension were used. Cell suspensions were generated by detaching the cells from one well as described for routine passaging and resuspending the pellet in 200 μl 1x PBS. For the detection of Ad5, AAV, and vaccinia virus, DNA was extracted according to the QIAamp DNA Mini Kit protocol (Qiagen, Basel, Switzerland) in combination with the extraction robot QIAcube (Qiagen) according to the manufacturer’s standard procedure. Lentiviral (HIV1)-specific nucleic acids (DNA and RNA) was extracted using the QIAamp Viral RNA Mini Kit (Qiagen) as described earlier (Bagutti et al., 2011).

Detection of Virus-Specific Genes and Human RNase P Control Gene in Nucleic Acid Extracts by Real-Time PCR

The following primers and probes were used: for the detection of Ad5: Ad5fiber-F (5’-aag cta gca ctt caa acatac-3’, at a final concentration of 100 nM), Ad5fiber-R (5’-ccc aag cta cca gta gca gta-3’, 300 nM), and Ad5fiber-FAM (FAM-5’-cct cac cac cac cga tag cag tac cct tca-3’, TAMRA, 100 nM) (Bagutti et al., 2011); for lentivirus (HIV1): HIV-1-PSS-F (5’-cgc agg act cgg ctg gta-3’, at a final concentration of 100 nM), Ad5fiber-R (5’-ccc aag cta cca gta gca gta-3’, 300 nM), and Ad5fiber-FAM (FAM-5’-cct cac cac cac cga tag cag tac cct tca-3’, TAMRA, 100 nM) (Bagutti et al., 2011).
nM), HIV-1-PSS-R (5'-gac gct ctc gca ccc at-3', 400 nM) and HIV-1-PSS-Pr (5'-FAM-cvc gtc ctc gct tgc ygt ggy ccc -TAMRA-3', 150 nM) (Bagutt et al., 2011); for AAV2: AAV-WT-F (5'-agt tga act tgt gtc ctc ggc tgt cta-3', 500 nM), AAV-ITR3-R2 (5'-cat cac gca ggc gct ata-3', 500 nm), and AAV-ITR3-VIC (VIC-5'-att aac ccc cca tgc tac ttg tct cag cc-3', TAMRA, 200 nm) for the detection of a fragment of the thymidine kinase gene of the vaccinia virus. 67'133-67'195); for MVA: VvI4L-F (5'-tcg atg aag gac agt tct ttc-3', 500 nM), and VvI4L-R (5'-ctg tta tct acg tag cc-3', 500 nM), and VvI4L-VIC (VIC-5'-agc agc cac ttg tac tac aca aca tcc gga-3', TAMRA, 150 nm) for the detection of a fragment of the ribonucleotide reductase I4L (GenBank® Acc.Nr. M35027: 67'066-67'290) and a 250-bp fragment containing the target region of the thymidine kinase TK gene (M35027: 84'017-84'267), respectively. Successful infection and integration of the transgene into the host genome were monitored by screening the cell monolayer (data not shown). To monitor the presence of Ad5 fiber gene sequences in the supernatant, samples were taken 5 hours (PI0) and 24 hours post infection (PI1) before washing. Thereafter, sampling of the supernatant took place on PI4, PI5, PI7, PI8, PI11, PI12, PI14, PI15, and PI17; Figure 1a). As judged by the presence of Ad5 fiber DNA sequences in the supernatant of Hela cells, the decrease of Ad5 until the cells started to detach (depending on the experiment before PI11, PI14, PI15, or PI17; Figure 1a). Less than 3% of the initial Ad5 genome copies number (PIO) could be detected on PI4. From PI5 onwards, including one cell passage, Ad5 DNA was detectable only in two instances (Figure 1a). As Ad5 mediates transient gene expression, Ad5-infected cells are often used for further experimentation before being passaged. Therefore, the study analyzed the process of washing on PI11 in more detail. Three different washing protocols prior to addition of fresh medium were compared: untreated, routine washing with PBS twice for a few seconds at room temperature or washing with medium twice for 5 minutes at 37°C each. Ad5 genome copies declined significantly from PI0 to PI3 (in absolute numbers approximately 10 7 genome copies/ml vs 10 6 and 10 5 for the respective titer, as well as judged by the ratio of Ad5-specific gene copies/ml of PI3 vs PI0; Figure 1b). The decrease of the decline was dependent on the titer used for infection, it was not influenced by the washing protocol. Ad5ΔE1GFP did not produce viral particles in Hela cells and clearance, therefore, could not be observed. However, the time and the degree of clearance were strongly influenced by the Ad5 titer applied. Consequently, it has to be investigated for each experimental setup of Ad5 infection before derogating the risk class. As proof of principle, Ad5ΔE1GFP (5 x 10 4 and 2 x 10 5 pfu/ml) was used to infect HEK293 cells, which are able to complement the deleted E1 genes of the replication-deficient Ad5ΔE1GFP, resulting in a steady increase of Ad5 fiber gene copies in the cell supernatant (Figure 1a). New viral particles were constantly produced until all cells were infected and eventually lysed after PI6. Thus, clearance could not be achieved.

**Results and Discussion**

**Adenovirus Type 5 (Ad5)**

Infection in Hela cells with Ad5ΔE1GFP (5 x 10 4, 2 x 10 5 and 2 x 10 6 pfu/ml) was confirmed by detection of the Ad5 fiber gene by real-time PCR after extraction of DNA from the cell monolayer (data not shown). To monitor the clearance of the supernatant, samples were taken 5 hours (PI0) and 24 hours post infection (PI1) before washing. Thereafter, sampling of the supernatant took place on PI4, PI5, PI7, PI8, PI11, PI12, PI14, PI15, and cells were passaged routinely up to four times (Figure 1a). As judged by the presence of Ad5 fiber DNA sequences in the supernatant of Hela cells, the decrease of Ad5 until the cells started to detach (depending on the experiment before PI11, PI14, PI15, or PI17; Figure 1a). Less than 3% of the initial Ad5 genome copies number (PIO) could be detected on PI4. From PI5 onwards, including one cell passage, Ad5 DNA was detectable only in two instances (Figure 1a). As Ad5 mediates transient gene expression, Ad5-infected cells are often used for further experimentation before being passaged. Therefore, the study analyzed the process of washing on PI11 in more detail. Three different washing protocols prior to addition of fresh medium were compared: untreated, routine washing with PBS twice for a few seconds at room temperature or washing with medium twice for 5 minutes at 37°C each. Ad5 genome copies declined significantly from PI0 to PI3 (in absolute numbers approximately 10 7 genome copies/ml vs 10 6 and 10 5 for the respective titer, as well as judged by the ratio of Ad5-specific gene copies/ml of PI3 vs PI0; Figure 1b). The decrease of the decline was dependent on the titer used for infection, it was not influenced by the washing protocol. Ad5ΔE1GFP did not produce viral particles in Hela cells and clearance, therefore, could not be observed. However, the time and the degree of clearance were strongly influenced by the Ad5 titer applied. Consequently, it has to be investigated for each experimental setup of Ad5 infection before derogating the risk class. As proof of principle, Ad5ΔE1GFP (5 x 10 4 and 2 x 10 5 pfu/ml) was used to infect HEK293 cells, which are able to complement the deleted E1 genes of the replication-deficient Ad5ΔE1GFP, resulting in a steady increase of Ad5 fiber gene copies in the cell supernatant (Figure 1a). New viral particles were constantly produced until all cells were infected and eventually lysed after PI6. Thus, clearance could not be achieved.

**Lentivirus (HIV1)**

HEK293T cells were infected with pLL3.7 lentiviral (HIV1) vector (at three different titers 10 3, 3 x 10 3, and 3 x 10 4 TU/ml). This construct does not contain any structural or replication-relevant genes; thus, it is incapable of replication. Successful infection and integration of the transgene into the host genome were monitored by screening the cell monolayer microscopically for the expression of GFP (Figure 2a). Five hours post infection (PI0) and prior to the washing step and medium substitution, control samples...
Clearance of Ad5: Ad5ΔE1GFP were used to infect HEK293 (a) and Hela (a, b) cells. The supernatant was analyzed for the presence of Ad5-fiber gene copies by real-time PCR at different time points post infection (days post infection, PI). The results are presented as mean ± SD. (a) Ad5ΔE1GFP titers used: 5 x 10⁴ pfu/ml (HEK; 2 Hela; 3 experiments), 2 x 10⁵ (Hela; 2 experiments) and 2 x 10⁶ (Hela; 2 experiments). Washing (PI1) and passaging steps (PI4, PI7, PI11, PI14) were performed immediately after sampling. (b) Ad5ΔE1GFP titers used: 10⁻¹, 10⁻², 10⁻³ dilutions of 2 x 10⁶ pfu/ml. On PI11 (after sampling) different washing procedures or no treatment (as indicated) were performed. Secondary axis on the right shows ratios of genome copies on PI0 to genome copies on PI4. Medium control (-). Limit of detection (LOD, 200 gene copies/ml); nd: not detected.
expression of viral GFP at least until PI14 in parallel without secretion of viral particles demonstrated stable integration of viral genome in the host cell genome (Figure 2a vs 2b). Derogation of the containment level of lentivirus (HIV1)-infected HEK293T is therefore practicable.

**Adeno-Associated Virus Serotype 2 (AAV2)**

Two different titers of wild-type AAV2 (ca. $10^4$ and $10^6$ genome copies/ml) were used to infect Hela cells (Figure 3). Two hours after infection of Hela cells, the medium was replenished and control samples were taken from the supernatant. As AAV2 did not express a marker protein visible by microscope, successful AAV2 infection was detected by analyzing AAV-ITR DNA copies in the host cells compared to those in the supernatant on PI1, PI6, and PI9 by real-time PCR (Figure 3a). To improve infection efficiency, etoposide was used in a second approach. The incorporation of AAV2-specific DNA sequences was confirmed and an increase of the ratio of AAV2 genome copies in the cell pellet to the supernatant was observed by up to $10^4$-fold on days PI6 and PI9. To monitor the clearance rate of viral genome in the supernatant, samples were taken on PI1 and thereafter on days PI4, PI7, PI11, and PI14 in each case before cell passage. AAV2-specific DNA sequences

**Figure 2**

Clearance of lentivirus (HIV1): HEK293T cells were infected with pLL3.7 lentivirus (HIV1) ($10^3$, $3 \times 10^3$, and $3 \times 10^4$ TU/ml). (a) Incorporation of GFP was analysed by fluorescence microscopy 24 hours (day 1) post infection (PI1), 7 (PI7) and 14 days (PI14) post infection (panel I). As a control, the respective phase contrast is shown (panel II). (b) The supernatant was analysed for the presence of HIV-Ψ-gene copies by real-time PCR at different time points post infection as indicated. Washing (PI0) and passaging steps (PI4, PI7, PI11) were performed immediately after sampling. The graph shows the results of 2 individual experiments per titer. Limit of detection (LOD, $257$ gene copies/ml); nd: not detected.
were still detectable in the supernatant even after four rounds of passaging (Figure 3b). Particularly, etoposide-assisted infections caused higher numbers and a longer persistence of AAV2 genome copies in the supernatant. AAV2 is only conditionally replicative. Several factors including cellular stress and superinfection can induce the release of viral particles (Berns & Giraud, 1996; Yalkinoglu et al., 1988). Since at least in the absence of etoposide no such factor was added, the authors’ finding contrasted with the replication-deficient characteristics of AAV2. Furthermore, Meyers et al. (2009) has reported rescue of AAV2 from differentiated keratinocyte in the absence of helper viruses or genotoxic agents. The cause of the persistence of AAV nucleic acids in the supernatant of the Hela

**Figure 3**

Clearance of AAV2: AAV2 was used to infect Hela cells in the presence or absence of etoposide as indicated. The supernatant (a, b) and cell pellet (a) were analyzed for the presence of AAV-ITR gene copies by real-time PCR at indicated time points post infection (days post infection, PI). (a) AAV2 titer used: 10 and 10^3 genome copies/ml, respectively. Secondary axis on the rights indicates ratio of genome copies integrated vs supernatant. One and two asterisks (**/***), respectively, indicate that gene copies were not detected in one or both duplicates. (b) AAV2 titer used: ca. 10^4 and 10^6 genome copies/ml, respectively, summarizing 2 experiments per titer. Passaging steps (PI1, PI4, PI7, PI11) were performed immediately after sampling. Limit of detection (LOD, 200 gene copies/ml); nd: not detected; eto: etoposide.
cells was not entirely clear in this setup and no distinct number of passages can be given for complete clearance.

**Vaccinia Virus (VV) and Modified Vaccinia Ankara (MVA)**

Infection of replication-competent VV leads to replication of the virus in the cytoplasm of host cells and subsequent release into the supernatant. This could be confirmed by infecting Vero-B4 cells with VVeGFP ($4 \times 10^4$ pfu/ml) and monitoring the supernatant for the presence of I4L gene copies by real-time PCR 5 hours post infection (PI0) as well as on PI1, PI4, PI5, and PI8 (Figure 4a). Over this period the cells were routinely passaged twice before they started to come off. An increase of VV-specific DNA in the

**Figure 4**

Clearance of VV and MVA: Vero-B4 cells were infected with (a) VVeGFP ($4 \times 10^4$ pfu/ml, 2 experiments) and (b) MVA ($2 \times 10^4$ and $2 \times 10^6$ pfu/ml, respectively, summarizing 4 experiments each titer). The supernatant was analysed for the presence of vaccinia I4L- (a) and TK4 (b) gene copies, respectively, by real-time PCR at different time points post infection as indicated (days post infection, PI). Passaging steps (PI1, PI4, PI7, PI11, PI14, PI18, PI21, PI25, PI28, PI32) were performed immediately after sampling. Limit of detection: 200 gene copies/ml; nd: not detected.
supernatant up to a factor of 3,000 could be observed. Therefore, no proper clearance effect was observed. Thus, downgrading is not possible.

MVA is a variant of VV whose replication capacity has been highly reduced (Meyer et al., 1991). Vero-B4 cells were infected with $2 \times 10^4$ and $2 \times 10^6$ pfu/ml of MVA. From PI1 onwards, the supernatant of the cells was sampled and analyzed for the presence of TK gene copies of MVA (Figure 4b). Subsequently, the cells were routinely passaged. MVA gene copies were detectable throughout the monitoring period at constantly increasing levels than used for infection (up to $8.4 \times 10^7$ gene copies/ml at PI11 vs $3.0 \times 10^5$ gene copies/ml at PI0) despite continuous washing and passaging. This suggested the production of MVA particles despite its much reduced replication capability. Although MVA is generally known to be highly attenuated, several cell lines other than CEF (chicken embryo fibroblasts) and BHK21 (baby hamster kidney cells) but including Vero-B4 have been shown to be semi-permissive (Okeke et al., 2006). At a titer of $> 10^7$ MVA gene copies/ml, Vero-B4 cells started to detach from PI18 onwards and eventually died off. This process was slow and lasted for up to 10 days and four passages (Figure 4b, series Vero-B4 2). Therefore, MVA clearance did not occur in viable cells.

Conclusion

The results of this study demonstrate that complete clearance could be achieved in individual experimental setups (Ad5 in Hela and lentivirus (HIV1) in HEK293T; Table 1) in contrast to other virus-host cell systems (Ad5 in Hek293, AAV2 in Hela, HEK293, VV and MVA in Vero-B4) as judged from the presence of vector-specific nucleic acids in the supernatant. Analyzing nucleic acids rather than infectious viruses undoubtedly lead to an overestimation of the amount of viral particles present in the supernatant (Higashikawa & Chang, 2001; Rohr et al., 2002). On the other hand, experiments could be performed with optimized infection rates on which the authors did not set priorities on. The degree and rate of clearance were influenced by several variables in the transduction setups, particularly vector titer and infection protocol. As this study has tested only one viral strain each, the type of strain and insert are likely to have an influence, too. In conclusion, procedures for clearance cannot be predefined for given virus-host systems, and the results presented can serve only as an indication. Each individual experimental setup will have to be tested for clearance before a safe derogation of the containment level will be possible.

Acknowledgments

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viral (HIV1) contaminations on laboratory surfaces as a tool for the surveillance of biosafety standards. *Journal of Applied Microbiology*, 111, 70-82.


Anthology of Biosafety XIII: Animal Production and Protection—Challenges, Risks, and Best Practices

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Anthology XIII is certain to capture your interest. Presentations from the February 2011 USDA-ARS 1st International Biosafety & Biocontainment Symposium “Animal Production & Protection: Challenges, Risks, and Best Practices” have been published here, which will forever record their knowledge and perspective on biosafety/biosecurity for the benefit of the biosafety community.

Researchers cannot tackle the problems of Q-fever, bovine encephalitis, monkey pox, or tularemia without working with the infectious agents. Computer models have their role, but the real research occurs in the field and in the laboratories.

Outbreaks of human and animal diseases and particularly outbreaks of food-borne illnesses do not respect political boundaries. Their impact, both in terms of health and economic devastation, is vast and immediate. The recent E. coli outbreak in Europe serves to remind us that people expect researchers to be able to quickly identify the cause, the source, and a cure all within a relatively short period of time.

Time issues create risk issues, and it is here where researchers, public health officials, veterinary health officials, and government authorities recognize that safety must remain a concern.

Just as the growth of biosafety knowledge and practices was paramount for conducting non-human primate research, the same need is true for agricultural research. The future availability of a safe and nutritious food supply is critical to social and economic development throughout the world. Diseases that seriously diminish the availability of food sources or that disrupt natural controls have far-reaching consequences. While research dutifully moves to find cures for diseases that haunt us, it is also essential that we not shortchange that which is most basic to our existence.

Contents include, in part: Introduction to Biocontainment and Biosafety Concepts as They Relate to Research with Large Livestock and Wildlife Species; From the Field to the Laboratory in an Animal Disease Outbreak Situation; Working with Biosafety 3 Agents That Interface Across Human, Livestock, and Wildlife Boundaries; Controlling Laboratory Risk...in a Global Biotech Revolution; Liquid Effluent Decontamination Design and Operations; Carcass Disposal for Biocontainment Facilities; and Strategies for Communicating with the General Public About High-containment Laboratories.
Survey of Biorisk Management in Clinical Laboratories in Karachi, Pakistan

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Abstract

This study was designed to survey the existing Biosafety and Biosecurity levels of the clinical laboratories in order to assess the controls in place to keep the laboratory workers, and the surrounding community, safe from an outbreak of infectious diseases in Karachi, Pakistan. The survey also helped to evaluate the present level of Biorisk management practiced in these clinical laboratories. Karachi was selected as the site of this survey because it is the hub of commercial activities in the region and has a population of about 25 million.

Place and Duration: This study encompassed a period of 8 months (January 2011 to August 2011) and covered laboratories in the 18 towns of Karachi, Pakistan. To begin the study, a survey questionnaire was developed with the help of Sandia National Laboratory (Albuquerque, New Mexico, USA). Results of the survey were disseminated to the relevant target audience and increased the level of information on these topics as well as ways to implement Biorisk management in accordance with CWA 15793-2008 (European Committee for Standardization, 2008). The results were also conveyed to the stakeholders (top management, laboratory management, laboratory managers, laboratory technologists, laboratory technicians, etc.).

This study reflects the current level of Biosafety practices in clinical laboratories in Karachi, Pakistan, where major gaps were identified in all the critical areas of Biosafety and Biosecurity, including risk assessment, standard microbiological practices, primary barriers/personal protective equipment, and laboratory design/secondary barriers, and select agent protocols. The gaps included the following:

- Biosecurity is not at all defined or understood.
- There is no protocol/guidance for reporting laboratory acquired infections (LAIs).
- There is no framework for registering these laboratories before commissioning.
- Limited or no training for Biosafety/Biosecurity is available on a continual basis.
- No regulatory body oversees Biorisk/Biosecurity activities or lab registration.

This study revealed that the degree of negligence regarding these requirements for clinical laboratories is very serious and work on an emergency basis is required to introduce, implement, and furnish Biosafety and Biosecurity guidelines. Training and literature in both English and the local Urdu language need to be developed and distributed. However, other extraordinary measures are also required because the majority of laboratories working at BSL-2 have no biosafety cabinets (BSCs), resulting in microbiological work being done on the open bench.

The main purposes of this study were (1) to develop a culture in which Biosafety and Biosecurity practices become routine in these laboratories; and (2) to commission a National Regulatory Body to define/dictate these guidelines and to ensure that new laboratories are registered before they are commissioned. In addition, this regulatory body will address the immediate registration of those laboratories already working.

Keywords

Biorisk Management, CEN Workshop Agreement (CWA) 15793-2008, Biorisk Assessment Models (BioRAMS), and Global Biorisk Management Curriculum (GBRMC)

Introduction

Laboratory services are an essential and fundamental part of all health systems. Reliable and timely laboratory tests are at the center of the efficient treatment of patients. Moreover, prevention and management of infectious and non-communicable diseases require accurate laboratory diagnostic information. Many therapeutic decisions rely heavily on data from health laboratories and, at the time of disease outbreaks or other public health events, laboratories are at the very heart of the public health investigation and response mechanisms. Today’s world cannot afford unreliable laboratory results, wasting precious time, precious samples, and too often, precious lives. Laboratories offer their services to many clients: patients, physicians, and public health programs for evidence-based decisions. Many medical, hospital, public health, and academic laboratories, be they public or private, contribute to health care and public health improvement through their diagnostic activities.

Introduction of the overarching “Biorisk management” approach that has resulted from careful thinking, comprehensive study of prevailing practices and recommendations, review of international norms and standards, and relevant ethical considerations, as well as learning about recent advances and trends in the fields of microbiology and pathology, has certainly turned attention to the level of competency in dealing with biohazardous agents in the laboratories, especially the clinical laboratories, in Pakistan. Unfortunately, at this time, not enough attention is paid to the safe handling and disposal of biological specimens and cultures coming for analysis and diagnosis that are capable of increasing the level of danger to the laboratory worker and simultaneously of spreading infection to the community and environment.

Introduction of the concept of “biological safety” has paralleled the development of the science of microbiology and its extension into new and related areas (tissue culture,
rDNA, animal studies, agriculture, and the field of biotechnology). The knowledge and skill obtained by microbiologists and pathologists to isolate, manipulate, and propagate pathogenic organisms required the parallel development of containment principles, appropriate facility design, and practices and procedures to prevent occupational infections in the biomedical environment or release of the organisms to the environment. The field of Biosafety promotes safe laboratory practices and procedures, and proper use of containment equipment and facilities. It also stimulates responsible activities among laboratory workers and provides advice on laboratory design. Increased laboratory work with biological specimens has greatly increased the risk of acquiring an infection, potentially rendering public and laboratory workers at greater risk of infection. This exposure and risk may be prevented or minimized by rigorous implementation of appropriate laboratory practices, safety equipment, improvement and maintenance of facilities, and formal validated training of technical, support, and administrative personnel. It is essential that personnel in laboratories where work with biohazardous agents is conducted are trained to a level of competence that ensures their safety and that of the community. For these reasons it is necessary to implement basic concepts in biological safety and to develop national codes of practice for the safe handling of pathogenic microorganisms in laboratories. This scientific community’s responsibility is to promote, coordinate, and disseminate knowledge with regard to basic concepts of Biosafety and to expand professional and public awareness through effective communication. With this perspective this study was designed to determine the current level of biosafety practice and laboratory design provided to laboratory workers to keep themselves and the environment safe from outbreaks of any occupational infection. This study was also instrumental in disseminating this knowledge to the target audience, and increased the level of information for all stakeholders (top management, laboratory management, lab managers, laboratory technologist/technicians, support staff, etc.).

Materials and Methods

From January 1, 2011 to August 31, 2011, labs in Karachi were surveyed with the help of one research associate, a research assistant, and the support staff. This study encompassed a period of 8 months and covered the 18 towns of Karachi (Table 1). The following organizations provided reference materials for this effort to comply with international policies for Biosafety and Biosecurity: The World Health Organization (WHO), the American Biological Safety Association (ABSA), and Sandia National Laboratory (Albuquerque, New Mexico, USA). A questionnaire

Table 1
Location of Laboratories Surveyed in Karachi, Pakistan

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<tr>
<td>Malir</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Bin Qasim</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Gadap</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>
was developed in consultation with international agencies and with an understanding of the local working conditions and financial constraints of laboratories in the different socio-economic zones of Karachi. The comprehensive survey questionnaire was composed of 76 questions highlighting different aspects of Biorisk management according to CWA 15793-2008 (European Commission for Standardization, 2008) and was administered in the laboratories in Karachi, Pakistan. Using the survey questionnaire that was developed for this study, these issues were addressed: What is the current culture of Biosafety? What is the level of awareness? What are the gaps with reference to:

- Risk assessment
- Safety practices/standard microbiological practices
- Primary barriers/personal protective equipment (PPE)
- Secondary barriers
- Biosecurity

The identification of stakeholders was a critical step. Karachi is divided into 18 townships which are subdivided into various union councils, each of which has scattered clinical laboratories in the form of private laboratories, private hospitals, public charitable organizations, and private entrepreneurs. The next step was the organization of many workshops to inform the owners of target labs about the study. This was done to overcome any resistance to future actions due to the results of the survey and to have access to these laboratories without any hindrance.

Results

Eighty-seven percent of the laboratories were clinical laboratories and 8% were associated with blood banks. The remaining 5% were both clinical and R&D labs (Table 2). The surveyed laboratory facilities were designated as district (based on the five districts in Karachi), regional, national, or international. As such, 84% were district level laboratories while 7% were regional, 7% national, and 2% international laboratories. Out of these 60 labs, about 70.8% were dealing with pathogens and about 92.3% were not handling any toxins because they were basically clinical diagnostic laboratories without any research responsibilities. The majority of laboratories were privately funded, although some were working in the public sector. About 90% of these facilities had an organizational chart. The survey revealed the size of these laboratories: 58.3% had 1-10 employees, and 41.7% had 11-50 employees. About 68.3% of these labs do not have arrangements to include students, postdoctoral fellows, trainees, or interns in their laboratory.

The survey revealed that the 60 laboratories surveyed demonstrated only a minimum level of awareness regarding Biosafety and Biosecurity. The reasons for this are multifactorial and are discussed in this article. The questionnaire was divided into two major categories, Biosafety and Biosecurity, and the results are discussed below under those headings.

Biosafety

The majority of laboratories, based on an analysis of their activities, should be BSL-2 labs; however, the survey results indicated that the activities were carried out at the BSL-1 level. In about 48% of these labs, permanent and temporary workers do not have access to biological materials since they were not performing any microbiological diagnostic testing and this service was not available in these laboratories. For microbiological culture and antibiotic sensitivity, specimens were sent to laboratories that have this ability. However, direct access to biological materials in labs with 1-10 employees was available in 42.6%, while 9.3% of labs with a staff of 11-50 had direct access to these materials.

The level of competency, based on the qualifications, training, and experience of the technical and management staff working in the laboratory, was found to be adequate in 80% of the surveyed laboratories, although all possessed local qualifications approved by their local government authorities. However, few training programs were provided by the laboratories for their employees. In addition, there were no opportunities for continuous professional education in 68% of the laboratories, with only 10% offering more than one training opportunity a year, 5% offering training opportunities annually, and 5% offering training every 2-3 years; 8.3% of the employees were taking training at their own expense (Figure 1).

Eighty-two percent to 83% of these laboratories do not have any procedures in place for the assessment of technical and professional performance and do not have any association with any professional organization. Only a small num-

### Table 2
Survey of Laboratory Activities

<table>
<thead>
<tr>
<th>Type of Facility</th>
<th>Labs Visited</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Laboratory</td>
<td>52</td>
<td>86.7</td>
</tr>
<tr>
<td>Research &amp; Development (R &amp; D) Laboratory</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Clinical Laboratory and Blood Bank</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>Clinical Laboratory, R &amp; D Laboratory</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Clinical Laboratory, Blood Bank and R&amp;D Labs</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>60</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
ber replied positively to the question ascertaining whether they are affiliated with national organizations such as the Infectious Disease Society of Pakistan (IDSP), the Pakistan Welfare Association (PWA), the Pakistan Association of Pathologists (PAP), or the Pakistan Biological Safety Association (PBSA). In terms of collaboration, it was mainly of a diagnostic nature because over 80% referred samples for special tests not available at their facilities. The R&D laboratories also had very little collaboration with national and international professional organizations.

The level of Biosafety management in place in these laboratories was not adequate, with 75% demonstrating no commitment to Biosafety and Biosecurity at their facility. In 21.6% of the labs, these plans were being developed, while about 3.4% had already developed clearly defined roles and responsibilities in this context (Table 3).

The surveyed laboratories have no formal training programs for Biosafety and Biosecurity and only a small number revealed any commitment to instituting training programs. But even those laboratories that showed a commitment to Biosafety and Biosecurity did not have documentation in place. It was alarming to note that there is no concept of having a full-fledged Biosafety Officer (BSO) in place, and only a small percentage considered such an employee important. Some of the duties associated with the role of BSO were assigned to different laboratory personnel for fulfillment of record and documents (Figure 2).

The survey also revealed that almost 91.7% have no standard operating procedures (SOPs) in place and no locally developed Biosafety manual for reference as per Bio-risk management standards (Figure 3). Appropriate biohazard signage was found in only 8.3% of the laboratories; in all instances, such signage was provided to these laboratories by the PBSA during its campaign for Biosafety and Biosecurity via seminars, workshops, and postings to PBSA members (Figure 4). The funds for these were provided by Med Path Laboratory and Diagnostic Centre (Karachi, Pakistan). An occupational health program was not implemented in 45% of these laboratories. However, the rest of the laboratories, although not aware of any protocols, were struggling to implement programs in partial fulfillment of this requirement.

**Figure 1**
Continuing Professional Education in Available to Workers in Survey Laboratories

**Table 3**
Management Commitment to Biosafety/Biosecurity in the Survey Laboratories

<table>
<thead>
<tr>
<th>Level of Biosafety Management is in Place at the Facility</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Management has not made a commitment Biosafety/Biosecurity at the facility</td>
<td>45</td>
<td>75.0</td>
</tr>
<tr>
<td>Management has stated a commitment to both and is working to implement it, but it is still in the process</td>
<td>13</td>
<td>21.6</td>
</tr>
<tr>
<td>Management has clearly defined roles and responsibilities for all aspects</td>
<td>2</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

N= No of Laboratories; %= Percentage
Figure 2
Assigned Roles and Responsibilities for Biosafety

Figure 3
Biosafety Manual in the Survey Laboratories

Figure 4
Risk Assessment Conducted in Survey Laboratories
The requirements for proper laboratory design were not fully implemented, and about 12% have no specifications for basic laboratory design criteria such as adequate illumination, properly secured shelving, and ease of cleaning. 30% of the laboratories have all of these requisites. In about 52% of these laboratories, no storage space was available. Most of the staff in these labs were working at BSL-1, and even if BSL-2 were available, very little time was spent working at this level.

Proper risk assessment was not done in most of the laboratories; only a few BSL-2 laboratories were following it. Only 1.7% were taking into account PPE, primary containment, and secondary barriers (Figure 4). However, the availability of equipment was limited. In addition, the majority of laboratories did not have any defined procedures for pre- and post-maintenance or any repair protocols written out. It was also alarming to note that there is limited primary containment and even if it exists in the form of a BSC, mostly it is non-functional or not used with safety intentions. Moreover, there was no certification authority to do that (Figure 5).

The availability of Biosafety Level 2 containment features, such as physical isolation, double door entry, adequate lighting, a fire protection system, a hand-washing sink near the lab exit, were available in only 25 of the laboratories surveyed. Karachi has hot spells for 6-8 months of the year; temperature is controlled by window units for air conditioning or, where windows are not present, through exhaust fans opening directly to outside. Ten percent of the laboratories had HEPA filtered exhaust, and, where present, it was through the biosafety cabinet. Only one laboratory had an HVAC system and it was a Mycobacterium tuberculosis laboratory operating at BSL-3.

Forty-nine percent of the laboratories (25) were not using PPE with the correct procedures in place. About 23 laboratories (40%) have limited procedures for PPE in place. Six percent of the laboratories have some PPE procedures in place but still lack oversight in implementation. Only 5% of the laboratories have an active PPE program, with well-defined procedures for donning, doffing, and maintaining PPEs. The types of PPE most often used include gloves, lab coats, goggles, masks, and face shields as shown in Figure 6.

Waste management is integral to complete Biorisk management, and it remains, to some extent, ignored by every laboratory surveyed. However, bagging and sending waste products to the public waste area is customary at 68.3% of the labs, while 12% add autoclaving before disposal. Eight percent incinerate wastes, while 5% send it to a City District Government, Karachi (CDGK) facility for incineration or to a landfill site provided by CDGK (Figure 7).

The work carried out at the surveyed laboratories is mainly with biological agents, with 60% not working with hazardous chemicals. However, 20% of the labs do work with and use flammable liquids and toxic chemicals.

**Biosecurity**

In 76.7% of the labs, no commitment to security was evident; however, 21.7% showed a partial commitment and 1.7% showed a strong commitment to security. No risk assessments regarding agent hazards, procedure hazards, and security were in place (Figure 8). In addition, no qualitative risk assessments had been performed to define the risks that a security system should protect against or to define a level of acceptable risk. Also, these labs evidenced no planning parameters or definitions for incident response planning. In 82% of the surveyed labs, no formal or informal training for Biosecurity was available. Ninety-five percent of the labs are situated on an open campus. Almost 90% of the laboratories have clearly defined perimeters, and intruders inside the facility are detected by guards and
in a few cases, by an alarm system or employees. In 10% of
the laboratories, the laboratory building is secured so that
only authorized or escorted personnel have access (e.g.,
doors are locked).

A badging system is employed in about 45% of the
labs, while 55% evidenced no formal badging system in
their facilities. In the approximately 45% of laboratories
providing a badging system for their employees, only 25%
of those badges contain ID photos. However, 12% do use
badges containing photos plus facility policy clearly pro-
tects badges from theft. About 7% of the labs used badges
containing photos and also reveal the employee’s level of
access. In about 2% of the laboratories, the badges hold
three levels of permissions (i.e., inclusive of access control).

It is interesting to note that there is no separate process
for hiring and or screening between nationals and foreign-
ers. The hiring process in 46.7%, of the laboratories con-
sists of interview with facility directors and verification of
professional records or certification. Thirteen and one-third
percent of the laboratories use interview process as the sole
hiring criteria; another 13.3% combine the interview pro-
cess, verification of credentials, skills testing, and other
factors as hiring criteria. Only one laboratory includes a
national criminal background check in the hiring process
(Table 4). There is no protocol for re-evaluation of person-
nel reliability or competence after hiring.

**Figure 6**
Biosafety Manual

![Bar chart showing percentages of laboratories with and without a badging system that covers all biosafety security.]

**Figure 7**
Waste Management in Survey Laboratories

![Bar chart showing percentages of laboratories using different waste management methods.]

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It is interesting to note that there is no separate process for hiring and or screening between Nationals and Foreigners. In case where hiring of new employees is done the following is self-explanatory.

This process is never rechecked in majority of cases and there is no such protocol for this in these laboratories. In a very small number of laboratories it is rechecked at specific intervals (e.g., once every 5-10 years if one stays for that period). In 88.3% of cases there is no protocol for contractual hiring of non-employees (i.e., Contractors and Students).
This survey also showed that 95% of the labs do not have the type of culture collection facility that contains large quantities of pathogens. The 5% that do have a culture collection are following proper procedures to secure it within the laboratory.

Generally, the reagent and equipment inventory is up-to-date at the surveyed laboratories, with the responsibility for pathogens, reagents, and equipment falling to the laboratory managers and administrators.

Almost 98% of the laboratories did not have any standard procedures for identifying, protecting, and/or disseminating information, including the handling, storage, transmission, and destruction of any sensitive information. No risk assessments exist that reference an information system; about 96.7% of the labs do not have any format for handling sensitive information. However, in a very few cases, the access to any information system is controlled by strong passwords and virus protection.

Discussion

Keeping in view that Pakistan only recently began acquiring knowledge about Biosafety and Biosecurity issues in a global perspective, the authors felt it was important to observe the current status of Biosafety and Biosecurity in this region and to begin its introduction and implementation as an international and national obligation to protect the world from emerging and re-emerging infectious diseases. Both “Biosafety” and “Biosecurity” are terms with which the global scientific community is becoming aware due to the integrated efforts of different non-government organizations (NGOs) that are introducing the terms and concepts to their local clinical and research laboratories. The purposes of this survey were to summarize the current levels of practicing Biorisk management in Karachi, Pakistan, to reveal any gaps in accordance with the CWA 15793-2008 standards, and to develop a strategy to fill in these gaps on a priority basis. This was a very robust survey and it was done in the spirit of bringing together different stakeholders working in different portfolios, private entrepreneurs, CDGK, semi-government organizations, NGOs, and laboratories working in the public sector. Unfortunately, the survey revealed that the gaps in standards are universal, and in some respects, exist in every aspect upon which the questionnaire touches.

This study revealed the gravity of negligence regarding the scientific disciplines of Biosafety and Biosecurity in clinical laboratories in Karachi, Pakistan. Work on an emergency basis is required to introduce, implement, and enforce guidelines. Training and literature in both English and in the local languages need to be developed and distributed and, in addition, extraordinary measures are required to do this as soon as possible because the majority of laboratories working at BSL-2 have no BSCs and are thus doing microbiology work on the open bench. The main directive identified by this study is to develop a culture that values Biosafety and Biosecurity practices as mandatory and routine in all laboratories and to have a regulatory body that defines guidelines and registers labs before they are commissioned. These steps must be implemented on an emergency basis within a stipulated, tight time frame.

Conclusions

This study reflects the current level of Biosafety and Biosecurity in clinical laboratories in Karachi, Pakistan. The survey revealed the following:

- Major gaps were identified with reference to all the critical areas of Biosafety and Biosecurity.
- Risk assessments, standard microbiological practices, primary barriers/personal protective equipment, laboratory design/secondary barriers, and select agent statements were all found to be lacking.
- Biosecurity is not at all defined or understood.
- There is no protocol/guidance for reporting LAs.
- There is no framework in place to register laboratories before commissioning.
- Limited/no training in Biosafety and Biosecurity is available on a continuous basis.

This information certainly draws attention to the seriousness of the situation in Karachi, Pakistan. To overcome this situation, the authors suggest that the following measures be taken in the coming years when research and development will be at full bloom in this region:

- Frequent training with respect to risk assessment, introduction to the Bioram program [Sandia National Laboratories], adherence to standard microbiological practices, usage and upkeep of PPEs and BSCs, institution of primary barriers, and the use of appropriate laboratory design (secondary barriers) for BSL-1 and BSL-2 laboratories
- Introduction of Global Biorisk Management Curriculum at local educational institutes
- Development of a Code of Conduct for clinical laboratory personnel
- Formation of a Pakistan Biosafety Regulatory Authority (PBRA) for certification/accreditation of laboratories
- Development of protocol for proper registration of laboratories
- Affiliation with professional organizations, such as PBRA, for future planning and discussion
- Implementation of Biosafety guidelines on an emergency basis at the national level
- Requirements for additional frequent trainings targeted to laboratory personnel
- Adherence to a focused vision for integrated Biorisk management with an emphasis on a commitment to change the current laboratory culture, to clarify the terminology, and to secure commitments from government officials who must be on board to ensure the success of this program.
- Implement a program to continuously increase capacity based on regional/country needs and establish accountability through development of country report cards.
Acknowledgments

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References


Knowledge and Awareness of Routine Biosafety Measures and Proper Waste Disposal Practices Among Healthcare Workers in Karachi, Pakistan

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1Jinnah University for Women, Karachi, Pakistan and 2University of Karachi, Karachi, Pakistan

Abstract

This study assessed the knowledge and awareness regarding routine biosafety measures and proper waste disposal practices among healthcare workers in Karachi, Pakistan, and also highlighted areas in which training was needed. To increase knowledge of biosafety and waste disposal requirements, the Virology & Tissue Culture Laboratory, Department of Microbiology, Jinnah University for Women, Karachi, Pakistan in association with the Biosafety Association of Pakistan (BSAP) executed a collaborative project entitled “Training Workshop on Proper Waste Disposal from Hospitals & Pathological Laboratories in Karachi, Pakistan,” with financial support from the Biosafety Association of Pakistan (BSAP) executed a collaborative project entitled “Training Workshop on Proper Waste Disposal from Hospitals & Pathological Laboratories in Karachi, Pakistan,” with financial support from the Biosecurity Engagement Program (BEP), the United States Department of State (DOS), and Civilian Research & Development Foundation (CRDF) for health-related issues in developing countries. A 50-question pre-workshop survey was administered to better understand the current biosafety and biosecurity measures in place at 250 state and private-sector clinical facilities (hospital and pathological laboratories) in the city. Participants from the 250 facilities were selected on the basis of first-come, first-served, but with preference for participants without prior knowledge of biosafety and biosecurity. After the training workshop, an evaluation survey was conducted with 28 participants. Results of that survey indicate that healthcare workers (HCWs) need to be offered training and refresher courses, in Urdu, to increase their level of knowledge and awareness of biosafety and waste disposal protocols to reduce both occupational exposures and the spread of infectious diseases in the community through improper disposal of infectious waste.

Introduction

Karachi is the major city in Pakistan, with about 7% of the total national population and 22% of the country’s total urban population. Since it is relatively more developed than adjoining regions, the city attracts a sizable number of visitors and immigrants who take residence for varying time periods to benefit from the healthcare facilities, educational institutions, and diversified job market. This is why most of the public and several private-sector healthcare facilities are over-utilized, resulting in an explosive increase in the number of diagnostic laboratories, blood banks, and medical facilities in the city. Although exact numbers are not known, these facilities regularly deal with potentially infectious agents and clinical waste without using proper handling techniques based on a risk assessment. Breaches in universal precautions are common. Also, although only 10%-25% of hospital waste is infectious and requires proper treatment and disposal (WHO, 2007), there is no segregation of waste products during collection, storage, or disposal. To address these facts, the Virology & Tissue Culture Laboratory, Department of Microbiology, Jinnah University for Women, Karachi, Pakistan in association with the Biosafety Association of Pakistan (BSAP) executed a collaborative project entitled “Training Workshop on Proper Waste Disposal from Hospitals & Pathological Laboratories in Karachi, Pakistan,” with financial support from the Biosecurity Engagement Program (BEP), the U.S. Department of State (DOS), and Civilian Research & Development Foundation (CRDF) for health-related issues in developing countries.

As a part of this project and also to complement the efforts of the Virology & Tissue Culture Laboratory, Department of Microbiology, Jinnah University for Women Karachi and BSAP’s Winning, Augmentation, and Renovation (WAR) for Biosafety in Pakistan initiative, a series of workshops to train healthcare workers and waste management personnel in Pakistan were initiated. This report is the outcome of the first Workshop/Certificate course in this series.

Methodology

This study was divided into three parts: 1) a pre-workshop clinical laboratory survey; 2) curriculum delivery; and 3) an end-of-the-workshop survey. Laboratories at which trained biosafety practitioners are already maintaining internationally accepted biosafety and waste-disposal standards were not included in the study. Instead, preference was given to those who were completely unaware of biosafety and biosecurity practices and needed education about personal safety, appropriate handling and disposal of medical waste, and community health.

1) Clinical Laboratory Survey

To make this effort a success, between January 11 and February 25, 2011, a 50-question, pre-workshop survey, designed using World Health Organization (WHO) standards, the U.S. Department of State Biosafety Engagement Program’s (BEP) guidelines (www.bepstate.net/), and the LOYOLA laboratory survey form (www.luc.edu/media/lucedu/ors/.../LOYOLA_LABORATORY_BS.do), was conducted to better understand the current use of biosafety
and biosecurity measures in Karachi’s 250 state and private-sector clinical facilities (hospitals and pathological labs). The questionnaire was divided into four sections based on standard microbiological practices, special practices, safety equipment, and laboratory facilities. Results of each section are discussed separately. All of the facilities should be Biosafety Level 2 laboratories, and survey results were used to develop the curriculum.

2) Workshop Curricula

The curricula of the workshop was divided into presentations to cover biosafety in the clinical setting, the importance of waste segregation—what it is and why do it?, understanding universal precautions and blood-borne pathogen safety, emergency response, spill response measures, occupational safety and health, biological safety cabinets (classes, types, safety use, certification), and operational laboratory biosafety practices. Three practical demonstration sessions on biological safety cabinets, personal protective equipment (PPE), and spill response were also provided to give hands-on training to the participants. In addition, all participants received a complete set of PPE along with samples of biohazard-labeled, autoclavable bags and hand sanitizers to help them introduce the materials and explain their importance to their organizations. The speakers addressed the topics in a clear and precise manner and were complimented by the participants during and after the workshop.

3) Participant Survey

The end-of-the-workshop survey was designed by BEP coordinators and was administered after completion of the training on waste disposal. It was divided into three sections: 1) satisfaction with the training program; 2) results and future plans; and 3) recommendations.

Results of Clinical Laboratory Survey

A) Standard Microbiological Practices

Sixty-five and one-fifth percent (65.2%) \((n=163)\) of the surveyed labs enforce restricted access when experiments are being conducted in the laboratory; 30% \((n=75)\) use gloves when working in the laboratory both for personal protection and to prevent the spread of infectious microorganisms; 85.2% \((n=213)\) of lab workers have a habit of washing hands during lab procedures; approximately 10% \((n=25)\) of the labs do not permit eating, drinking, and applying cosmetics; 45.2% \((n=113)\) do not use mouth pipetting techniques; 32.2% \((n=83)\) have policies for the safe handling of sharps; and 6% \((n=15)\) have instituted precautions to prevent the creation of aerosols. Seventy percent \((70%) \((n=175)\) of the labs decontaminate work surfaces daily, while 22% \((n=55)\) decontaminate cultures, stocks, and other laboratory wastes before disposal. Insect or rodent control programs are in effect in 70% \((n=175)\) of the labs in the city.

B) Special Practices

Of the 250 laboratories, 85.2% follow required BSL-2 precautions such as: 1) closing lab doors during experiments; 2) having access to the laboratory controlled by the lab director; and 3) restricting persons who are at increased risk of infections from entering the labs. Out of 250 laboratories, 87.2% \((n=218)\) of lab directors establish policies and procedures for handling potential biohazards, 53% \((n=133)\) have a hazard warning sign posted; and 10% \((n=25)\) regularly immunize the laboratory professionals against agents handled in the labs. Serum samples are collected and stored for all laboratory staff or persons at-risk in approximately 1% \((n=3)\) of the labs. About 5% \((n=13)\) have an approved biosafety manual including preparations and standard pro-

### Table 1

Example of Pre-workshop Survey on Standard Microbiological Practices in the Laboratories in Karachi (Section A).

<table>
<thead>
<tr>
<th>Question No.</th>
<th>Question</th>
<th>Positive Answers ((N=250))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Is access to the laboratory limited or restricted?</td>
<td>163 (65.2%)</td>
</tr>
<tr>
<td>2</td>
<td>Are gloves worn during routine laboratory work?</td>
<td>75 (30%)</td>
</tr>
<tr>
<td>3</td>
<td>Do laboratory procedures include washing of hands?</td>
<td>213 (85.2%)</td>
</tr>
<tr>
<td>4</td>
<td>Are eating, drinking, smoking, handling contact lenses, and applying cosmetics prohibited?</td>
<td>25 (10%)</td>
</tr>
<tr>
<td>5</td>
<td>Is mouth pipetting prohibited?</td>
<td>113 (45.2%)</td>
</tr>
<tr>
<td>6</td>
<td>Are policies for safe handling of sharps in place?</td>
<td>83 (32.2%)</td>
</tr>
<tr>
<td>7</td>
<td>Are careful procedures in place to minimize the creation of aerosols?</td>
<td>15 (6%)</td>
</tr>
<tr>
<td>8</td>
<td>Are work surfaces decontaminated at least once a day or after a spill?</td>
<td>175 (70%)</td>
</tr>
<tr>
<td>9</td>
<td>Are regulated wastes decontaminated before disposal by an approved method?</td>
<td>55 (22%)</td>
</tr>
<tr>
<td>10</td>
<td>Is an effective insect and rodent control program in place?</td>
<td>175 (70%)</td>
</tr>
</tbody>
</table>
cured; 3.2% (n=8) of lab workers receive appropriate training on the hazards in their laboratory. Only in 10% (n=25) of the labs does the director ensure that lab personnel are proficient in microbiological practices and techniques and operations specific to working in a BSL-2/3 lab. Fifty-six percent (56%) (n=140) are aware that they should be very careful when handling sharp instruments and that glassware should be substituted with plasticware. Nine percent (9%) (n=23) of labs use needle-locking syringes or syringe-needle units for injection or aspiration of fluids, and 7% (n=18) of labs use needleless systems and other safety devices. Eight percent (8%) (n=20) of labs follow the proper procedure for disposing of broken glassware mechanically using tongs and forceps. Approximately 67.2% (n=168) of the labs decontaminate lab equipment using an effective disinfectant; 2% (n=18) properly decontaminate spills involving infectious materials; and 10% (n=25) decontaminate equipment and materials before removal from the lab. Cultures, specimens, and body fluids are placed in proper leak-proof containers during work and transportation in 90% (n=225) of labs. Spills and accidents that could result in exposure to infectious materials are reported to a director who evaluates them, provides treatment, and maintains records in 75.2% (n=188) of labs.

C) Safety Equipment (Primary Barriers and Personal Protective Equipment)

Only 2% (n=5) of labs use biological safety cabinets when working with infectious material. If work with infectious materials is not conducted in biological safety cabinets, then a combination of PPE such as gowns, gloves, masks, and shields are used in 8% (n=20) of labs. About 5.2% (n=13) of lab workers wear wraparound gowns, scrub suits, or coveralls, and reusable clothing is decontaminated regularly. Thirty percent (60%) (n=150) of lab workers wear gloves when handling infectious materials. Frequent changing of gloves accompanied by hand washing is followed in 6% (n=15) of labs. Respiratory or face protection (masks) is used in rooms containing infected animals in 5% (n=13) of the laboratories.

D) Laboratory Facilities (Secondary Barriers)

Restricted access and self-closing doors are present in 1.2% (n=3) of labs. Four percent (4%) (n=10) of labs contain a hand-washing sink near the door. Interior surfaces of walls, floors, and ceilings are washable, smooth, impermeable to liquids, and resistant to chemicals and disinfectants, and any spaces between doors and frames are sealed in 72% (n=180) of labs. Ninety-eight percent (98%) (n=245) of labs have impervious bench tops that are resistant to moderate heat, organic solvents, acid, and alkalies. Approximately 83.2% (n=208) of labs have lab furniture capable of supporting the anticipated load of equipment on them and are accessible for cleaning; chairs and furniture are covered with non-fabric material that is easily decontaminated. Eighty-nine percent (89%) (n=224) of all labs have closed or sealed windows. Biosafety cabinets are present in 4% (n=10) of labs. Three percent (3%) (n=8) of labs have an eye-wash station and 76% (n=190) have adequate illumination for all activities.

Discussion

Although the Lab Director establishes policies and procedures for potential biohazards and biosafety measures, this study revealed that those policies were not comprehensive or implemented consistently in Karachi, Pakistan. Eating, drinking, and applying cosmetics in the laboratory are very common, but these practices were prohibited in only 25 (10%) of the labs (Table 1). Although gloves are worn in 30% of the laboratories, frequent changing of gloves is practiced in only 13%-15% of laboratories, while safe sharps practices are employed in only 32% of the facilities. An eye-wash station, biological safety cabinets, and some of the PPEs are used in only 3%, 4%, and 8% of the facilities, respectively.

Procurement of proper PPE for laboratory staff is difficult in Pakistan because there is an insufficient number of distributors from whom the products can be purchased. This is because stakeholders do not sufficiently understand the significance of PPE, professional input is absent, and facility managers often exhibit a general work slackness and inability to deal with these issues. Although two major hospitals, both in the private sector and both following biosafety and biosecurity measures regarding waste management, are importing PPE from international markets on their own, the rest of the hospitals and laboratories are dependent on the limited PPE supply of the local markets.

Seventeen (17) questions were asked in the end-of-the-workshop survey conducted by BEP coordinators, and the response rate was 100% (that is, all 28 selected participants gave their response willingly). Training topics such as waste segregation, blood-borne pathogen safety, spill response, and operational biosafety practices were welcomed. Participants stated that the training provided opportunities to exchange ideas and that the logistics of the training workshop and its overall organization were very good. More than 90% of the participants responded that their overall knowledge was increased after completing the course. Almost 95% of the participants said that they will share the information and knowledge gained at the workshop with their staff and colleagues, and will also initiate joint projects to implement new strategies for proper waste disposal. Overall satisfaction with the training program and the competence of the coordination team were rated very well by a majority of those surveyed.

Emphasis on Segregation and Proper Treatment of Hospital Waste

Not all healthcare waste is biohazardous; infectious waste and sharps constitute approximately 10%-25% of the healthcare waste. (El-Sharkway, 2009; Yanez et al.,
However, if this infectious waste is not disposed of properly, it could contaminate the normal waste, rendering it biohazardous material too (International Healthcare Waste Network, www.who.int/water_sanitation_health/medicalwaste/002to019.pdf). In 2007, the Pakistan Medical Research Council (PMRC) conducted a cross-sectional, hospital-based survey (Fazil, 2009) with 137 personnel involved in hospital waste management at 68 randomly selected healthcare facilities in urban areas in Karachi. Data from 137 sanitary workers at 9 hospitals, 11 maternity homes, and 29 dispensaries revealed that knowledge about hospital waste management was inadequate in 100% of the workers. Additionally, the study further showed that 25.5% of the healthcare facilities disposed of infectious and non-infectious waste, without any treatment, into public dustbins, and 73.7% of the sanitary workers carried waste in open buckets for final disposal. None of the sanitary workers had undergone routine medical check-ups except when ill and 67.9% were not provided any protective equipment. It was also reported that 96.4% of participants were not interested in health education about the safe disposal of healthcare facility waste; rather, they were just doing their jobs for the salary.

Another study conducted on the same issue in 2005 in eight teaching hospitals (>200 beds) in Karachi highlighted the routine hospital waste management practices including segregation approaches, storage arrangements, and collection and disposal systems (Rasheed et al., 2005). This study revealed that out of eight hospitals visited, two (25%) were segregating sharps, pathological waste, chemicals, infectious, pharmaceuticals, and pressurized containers at the source. For handling potentially dangerous waste, two (25%) hospitals provided essential protective gear to their waste handlers. Only one (12.5%) hospital regularly offered training sessions to its waste handling staff. Five (62.5%) hospitals had storage areas, but they were generally not protected from access by scavengers. Five (62.5%) hospitals disposed of their hazardous waste by burning it in incinerators, two (25%) disposed of their hazardous waste at municipal landfills, and one (12.5%) was burning waste in the open air without any specific treatment. No record of waste quantities and disposal method was generally maintained. Only two (25%) hospitals had well documented guidelines for waste management or a proper waste management team (Rasheed et al., 2005). Today, in 2012, the scenario has not improved much. Local municipal vehicles collect medical waste on a daily or weekly basis, depending upon their contract with the hospital/laboratory administration, and most dispose of it without adhering to acceptable guidelines. Hospital and laboratory staffs (e.g., nurses, paramedics, and janitorial staff) still do not appear very much interested in health education regarding the safe disposal of healthcare facility waste.

On the whole, the knowledge and awareness of biosafety and biosecurity issues around waste disposal by HCWs in Karachi are far below a desirable level due to inadequately trained staff. This is a major impediment to the creation of a proper system, which was found lacking in almost all public and most of the private hospitals and laboratories. Healthcare waste from hospitals and laboratories located in slum areas or the low socio-economical areas of the city is either dumped at community waste sites “kuchra kundis” or sold directly to junk dealers—“kabaris.” Scavengers, driven by extreme poverty and ignorant of risks, are involved in sorting and handling the contaminated materials at community waste sites; their favorite items for resale include syringes, infusion sets, empty bottles, and blood bags. Scavenger boys and sweepers at healthcare facilities also sell these goods to junk dealers. Unfortunately, a market for healthcare waste exists in the recycling business. Moreover, the plasticware industry remains the biggest buyer of used syringes, infusion sets, and blood bags (Altat & Mujeeb, 2002). Most of the used syringes are recycled into plastic items, but some are washed and simply packaged for resale. Unfortunately, the public cannot differentiate between new sterilized syringes and recycled unsterilized syringes (Ahmad, 2004). It is important to note that the Ministry of Environment in consultation with the Environmental Health Unit of the Ministry of Health, Pakistan has designed guidelines for hospital waste management in Pakistan; these guidelines are incorporated into the Hospital Waste Management Rules 2005 and the National Sanitation Policy 2006 which cover all aspects of safe hospital waste management, including the risks associated with the specific types of waste; forming waste management teams and their responsibilities; collection, segregation, transportation, storage, and disposal methods for various waste categories; and, above all, supervision, monitoring, and evaluation of the entire process. Additionally, the National Program for Prevention and Control of Hepatitis is also in the process of developing national guidelines. This program has also developed Trainer and Trainee Manuals for various categories of healthcare workers including health managers, professionals, and waste management staff (Fazli, 2009).

**Conclusion**

This study was an attempt to re-examine the current status of the biosafety programs for clinical laboratory staff and the medical waste issues in Pakistan. On the whole, the situation is not very promising. The end-of-the-workshop survey questions related to level of awareness before, and after, introduction to PPE, concepts of biosafety and biosecurity, use of basic biosafety measures, etc. The participants, who were selected employees from the hospitals and laboratories, were found more aware about personal safety, waste segregation, and safe sharps practices after the workshop. It was also noted that many of the participants had not even seen the different kinds of face masks or labeled/unlabeled autoclavable biohazard bags and were not able to distinguish between simple polyethylene disposable gloves,
surgical gloves, and examination gloves before the workshop. More than 90% of participants requested more trainings and appreciated the workshop organizers for conducting such a valuable training course.

Raising awareness through organized campaigns among stakeholders is a key suggestion. Training sanitation and healthcare workers, providing essential props, separating infectious waste from general waste at the source, transporting waste safely, procuring and installing incinerators, separating harmless and non-hazardous recyclables, and developing healthcare waste management plans are some of the post-workshop recommendations. The authors are also interested in organizing the same kind of workshops for the HCWs from other cities in the country. These hands-on training workshops increase awareness about biosafety, biosecurity, and personal safety regarding infectious medical waste. Every member of the community has the right to be informed about potential health hazards, so members of the professional community have a responsibility to share the burden of providing this education.

The authors were not surprised by the survey results, as use of personal protective equipment is not practiced in the majority of the facilities (89%) surveyed. About 95% of the hospitals (majority located in moderate- to low-socio economic areas of the city) dump their waste openly inside or outside the premises without proper prior autoclaving or disinfection treatment. According to this survey report, local municipality vehicles collect that waste either on a daily or weekly basis, depending upon the contract with the hospital administration. Properly designed storage areas are a luxury in 90% of these facilities. Most of the healthcare workers, especially laboratory personnel, complained that they were not provided with the gloves and masks while handling infectious waste. Only 20 (8%) of the laboratories out of the 250 surveyed had biosafety cabinets and well maintained waste management plans. On the whole, the authors conclude that healthcare waste management and close monitoring are not the only solution; increasing the social expectation for mass education, increased motivation, and a firmer sense of ethics among both the stakeholders and the HCWs will reduce the risk of steadily increasing deadly infections in the society. Janitorial staff, who are at the highest risk for infections during waste disposal, must be trained properly in the local languages since most cannot understand English. The Workshop/Certificate course described in this article was the first step towards this goal. The authors hope to continue these efforts to best serve the community.

**Acknowledgments**

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**References**

Do you have a biosafety question and you’re not sure who to ask? Send your questions to the “Ask the Experts” column and we get them answered for you. Please e-mail your questions to Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Final Musings of an Old Biosafety Professional

Several years ago, I wrote an article for the “Ask the Experts” column entitled “Musings of an Old Biosafety Professional.” As I sit down to write my last column, I’d like to reflect on the state of the art as I see it today. Yes, my last column for “Ask the Experts.” I’ve contemplated giving up this column for over a year now because I find that my professional work load has increased to the point that I am not able to spend the time that I need to spend on preparing good informational columns for each issue of the journal. My feeling is that when you can’t do it well, you shouldn’t do it at all.

Over the years, many of the questions that have been addressed in this column have come from “new” biosafety professionals who are somewhat inexperienced and are trying to cover all the basics to insure the safety of their fellow employees and to insure that they are following the “rules” (i.e., regulations) that have been imposed by Federal and State regulators. My advice is to not be overly concerned with what others are doing, but rather to read and understand the regulations and determine how you can meet the intent of the regulations, at your facility, in the most cost-effective and efficient manner. The regulations regarding biohazards are generally relatively easy to follow as long as we do not make them more difficult than we need to. I have found that many of the problems that we face with regulations come directly from our misunderstanding of the regulations, or from our failure to realize that biohazards are different from other hazardous materials. We try to apply either radiation hazard or chemical hazard solutions to biohazardous situations. This doesn’t make sense. Know the difference and apply your knowledge of biological material to developing a solution. Be willing to fight for your solution, even with the regulators.

Another problem we face is that of public perception. I am continually amazed at how easy it seems to be to give in to public perception and do things because someone, who doesn’t really understand the science, says there is a problem. I believe that some of this philosophy comes from our failure as biosafety professionals to adequately explain the facts or from our predilection to ignore someone’s concern because it really doesn’t make sense to us that they would be concerned. It is also much easier, early in an argument, to just give in and do what you are told. If the public is concerned, this is a legitimate concern whether it makes sense scientifically or not. We need to address these concerns logically and with empathy. I would encourage you to know your science and, when asked, explain it in detail. Don’t be confrontational; a calm, logical explanation can go a long way toward defusing a potentially disagreeable situation.

In monitoring the ABSA e-mail group “biosafety,” I’ve noticed many “How do you do it at your institution?” questions. While getting a feel for how others are doing things may be helpful, don’t trust all of your associates to know how to address your particular problem. Each facility is different and the problems associated with a particular facility are unique to that facility. Listen to advice and then apply it to your situation appropriately. There is no “one size fits all” answer. There is no substitute for evaluating the real problem and developing a workable solution for that unique problem.

In a similar fashion, be wary of the researcher who wants things done the way it was done at a prior institution. While it is important to work with the research staff on safety matters, what they did somewhere else does not necessarily constitute good practice at the new facility. The facility may be engineered differently or the management of the facility may have different requirements. Local regulations may allow for changes or may restrict the way things are done. Biosafety professionals should have the ability to question new researchers’ protocols and have the authority to insure that they meet the requirements of the new facility.

A pet peeve of mine is what I perceive as the excessive use of PPE in biocontainment laboratories. I would ask that you consider the real need for PPE in a biocontainment laboratory. The OSHA booklet Assessing the Need for Personal Protective Equipment: A Guide for Small Business Employers (OSHA, 2000) states that the preferred way to protect personnel from exposure to workplace hazard is “…through engineering controls or work practice and administrative controls, but when these controls are not feasible or do not provide sufficient protection, an alternative or
supplementary method of protection is to provide workers with personal protective equipment (PPE) and the know-how to use it properly.” There is a need to assess the probability of exposure as a result of working in a potentially hazardous environment or with a potentially hazardous work practice, and, whenever possible, a requirement to engineer out the problem (recognizing that engineering solutions never result in zero risk) or to change the practice. If neither of these options provides a solution, then PPE should be used. The OSHA guide (OSHA, 2000) also states that: “Employers should institute all feasible engineering, work practice, and administrative controls to eliminate or reduce hazards before using PPE to protect employees against hazards.” and indicates that employers should “...choose the appropriate PPE for protection against that hazard.” (Emphasis added). Understand that PPE is sometimes necessary, but that wearing PPE may result in a worker becoming fatigued, stressed, or encumbered to the point of losing concentration for the job-at-hand. Inappropriate use of PPE may, in some instances, be as dangerous as the hazard from which you are trying to protect the employee.

Finally, it comes to my attention that a lot of the things we do to ostensibly protect personnel and the environment from biological hazards are done “because they have always been done that way.” Biohazard science and the methodologies that we use in laboratories have changed since the old “Fort Detrick” days. Engineering has vastly improved. We have become much more sophisticated in our approach to containment. It is time that we think outside the box and develop appropriate controls based on current knowledge and current research in containment. Encourage practical biosafety research into the solutions for recognized problems. Don’t ever rely on what someone tells you is the best solution to a particular problem, just because they say it works. ASK FOR THE DATA. Critically review the protocols and look to yourself to determine if the research on the product or procedure really answers the questions and proves that the results demonstrate what is claimed.

So, that’s it—the final musings of an old biosafety professional. Oh, you will still hear from me as I read the ABSA e-mail group “biosafety” and find myself saying, “Okay, let’s get back to basics.” I will always remember, and encourage you to remember, the words on the UNC Class of 1981 t-shirt “Biohazard Scientists do it with Control.” Keep on controlling.

Reference

Animal Bytes
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Animal Bytes examines biosafety challenges posed when conducting work with animals and provides solutions that promote both safe and responsible research. Good safety and animal husbandry are essential for good science. Learn about best practices when working with animals and applied safety information that can be used every day. Please e-mail your comments, questions, and insights to barbara_johnson@verizon.net or to Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Lentiviral Vectors and Animal Biocontainment Levels for Studies with Mice

Introduction to Lentiviral Vectors
Since the late 1990s, retroviral vectors have gained increasing popularity as gene delivery systems. Lentiviruses or “slow viruses” are a subclass of retroviruses that are able to infect both proliferating and non-proliferating cells. Because they integrate into the host cell genome and have powerful promoters, lentiviruses are able to deliver genes to target cells with high efficiency and induce long-term, stable expression of the introduced gene of interest. While lentiviruses infect many species, those which have been used as viral vectors include Feline Immunodeficiency Virus (FIV), Simian Immunodeficiency Virus (SIV), and most prominently Human Immunodeficiency Virus (HIV). The focus of this article is animal biosafety considerations for HIV-based lentiviral vectors in mice.

Inherent occupational hazards associated with lentiviral vectors include the ability of the virus to become competent to replicate in host cells and cause oncogenesis. The following is a very brief overview of how commercially available lentiviral vector systems have evolved to reduce these hazards. HIV-based lentiviral vector systems, referred to as “first generation,” were developed to increase the viral titer by increasing the range of cells that could be infected. To
create the first generation system, the envelope genes from wild type HIV were typically replaced with envelope genes from vesicular stomatitis virus (VSV). The 3-plasmid construct allows for insertion of the gene of interest, and in theory allows for only one round of virus replication. However, during replication the virus can recombine in such a way that replication-competent retroviruses (RCRs) are generated and pose a hazard as the virus and gene of interest can now continue to replicate unchecked within cells. To further reduce the risk of forming RCR, second-generation vector systems were created by deleting more genetic elements (accessory genes) from the virus. This was an improvement but when assayed, RCR could still be detected. Third-generation systems made additional deletions to the virus genome (removed the tat gene) and newer, more improved systems made alterations such that the vector and packaging system were separated on 4 plasmids instead of 3 plasmids, to further reduce the probability of recombination.

In a recently published article, Reuter et al. (2012) measured viral vector shedding in mice following intravenous inoculation with either third-generation replication-incompetent lentiviral vector, an adenoaviral serotype 5 vector, or a recombinant adeno-associated viral vector. For the purpose of this column, only information pertaining to lentiviral vectors will be summarized. Environmental stability tests on fomites showed infectious lentiviral vector was recoverable after deposition on dry plastic for 24 hours, and in vector-spiked soiled bedding for up to 72 hours. Infectious virus could also be recovered by swabbing the injection site (tail) for as long as 24 hours, though no infectious virus was detectable in the soiled corn cob bedding in the inoculated group-housed mice. Positive tail swabs were attributed to vector leakage when removing the needle from the injection site. No viral nucleic acids were detected in blood, urine, or fecal samples taken on days -1, 1, 3, and 7 (relative to inoculation) from inoculated mice or in co-housed non-inoculated mice (lower limit of detection for the assay was 200 IU). While the results of this study indicate that risks associated with shedding are low, the authors caution that increased exposure risk is possible if there is a pre-existing viral load/infection of the animal or laboratory and animal care staff.

Factors to Consider in Assessing Risk When Using HIV-based Lentiviral Vectors in Mice

The simplest approach is to identify and characterize the elements that pose risks such as:

- The type of vector system used (i.e., increased risk with a higher potential for replication competence)
- The gene of interest that has been inserted (i.e., increased risk if an oncogene)
- The host animal (i.e., increased risk if a permissive host, one that supports virus replication)
- The vector quantity and titer (i.e., increased risk with higher titer/quantity)

Replication Competent Lentivirus (RCL) Testing

RCL testing is required by the FDA for all lentiviral stocks used in human clinical trials. RCL testing is also desirable for safety data in laboratories not involved in human clinical trials. However, RCL testing requires specialized expertise and assays that may not be available in every lab. The NIH recognizes that handling positive controls during RCL assays may put staff at increased risk of exposure in labs that do not routinely work with lentiviral vectors (NIH, 2011). In these cases, the IBC can conduct a risk assessment of the vector system used and the data generated from past work with the system to assign an appropriate biocontainment level and identify appropriate practices and PPE.

Containment Levels Associated with Wild Type Mice and Mice with Human Xenograft Tissue

The IBC and IACUC should review and approve procedures and equipment plans for the initial containment of animals post-inoculation, decontamination of inoculation sites and soiled caging, and the transfer of animals to a lower containment level. Primary containment PPE is commensurate with ABSL-1 or -2 recommendations and the risk assessment for the work being conducted (U.S. Department of Health and Human Services, 2009).

Questions often arise regarding the appropriate animal biocontainment level for conducting work with established 3- and 4-plasmid third-generation HIV-based lentiviral vectors in mice. Replication of HIV is not supported in wild type mice (i.e., mice that do not express a genetic mutation or manipulation such as transgenic or knockout that could theoretically support virus replication). Hence, the risk of shedding is greatly reduced and associated primarily with the inoculum itself. In wild type mice, current best practices recommend that the initial delivery of the vector be conducted under BSL-2 containment using safe-needle systems (i.e., retractable sharps or other engineered devices) as this can potentially infect humans. Mice containing human xenograft tissue and mice injected with human cells infected with lentiviral vectors are a permissive host for the virus, allowing the replication and shedding of HIV-lentiviral vectors. Commonly, these mice are maintained at ABSL-2 or ABSL-2 enhanced.

Enhancements may include use of primary containment such as conducting cage changes in a BSC, use of filter-top cages or negatively ventilated IVC racks, minimizing the use of sharps wherever possible, and conducting other rodent manipulations in the BSC. If a risk of splash or aerosolization of liquids exists, a face shield and mask are used to prevent mucosal or ocular exposure.

In March 2006, the NIH RAC convened a meeting on biosafety considerations with lentiviral vectors and recommended that animals be initially housed in ABSL-2 containment for 1-7 days, then moved to standard ABSL-1 containment provided the inoculation site has been cleaned and the bedding changed (NIH, 2011). An Internet search was conducted on best practices among a variety of institu-
ions to identify alternative recommendations. Three institutions (Emory University, Oregon Health & Science University, and Stanford University) were found to have different approaches for housing wild type mice following inoculation. Each method provides flexibility for the type of containment space available and employs various safety procedures and/or the use of primary containment equipment for housing animals post-inoculation.

1. Emory University allows inoculated animals to be held in filter-top cages and IVC racks in demarcated areas of an ABSL-1 room. Specific signage/labeling is needed on each cage stating “ABSL-2 Biohazard Containment—Quarantine for Lentiviral Vector Research.” Seventy-two hours post-inoculation, animals can be transferred to a clean cage and housed in ABSL-1 containment.

2. Oregon Health & Science University permits animals following inoculation to be housed in standard ABSL-1 containment. The animals’ inoculation sites are disinfected immediately with 70% ethanol. Animals are temporarily placed in a holding container devoid of bedding until the sites are completely dry. The animals can then be moved to a standard ABSL-1 containment cage, and the holding container is decontaminated with 70% ethanol.

3. Stanford University requires animals to be initially housed in ABSL-2 containment. If the inoculation site has been cleaned and the bedding changed, the animals may be moved to standard ABSL-1 containment after 48 hours.

The Need for Biosafety Data: Looking at a Path Forward

The literature contains a paucity of data regarding viral vector shedding and migration in laboratory animals. However, this information is essential to assess exposure hazards and make informed decisions on appropriate biocontainment in mice inoculated with lentiviral vectors, or for that matter, other commonly used viral vectors. At risk is the health and safety of animal care personnel and research staff, and the potential for accidental exposure to viral vectors of other animals in the vivarium. The lack of scientific data on viral vector shedding and many other topics related to animal biosafety and biosafety in general hampers the ability to accurately assess and take appropriate steps to mitigate risks. The lack of research data is a reflection of the extremely limited funding available in the form of grants and other resource streams for applied and basic biosafety/animal biosafety.

There is some good news. The American College of Laboratory Animal Medicine (ACLAM) has a grant program where researchers can submit a letter of intent and apply for a grant to address knowledge gaps across a variety of topics including animal biosafety. As this column is going to print, ACLAM has issued a Request For Proposals to challenge the need for 10-15 air changes per hour in animal housing rooms and to develop rational HVAC standards for modern facilities and those using individually ventilated caging. The impact this type of research may have on safety, program sustainability through reduced operating cost, and development of animal housing environmental standards could be significant. The American Association for Laboratory Animal Science (AALAS) sponsors Grants for Laboratory Animal Science (GLAS) which provide competitive short-term research funding in the laboratory animal science field. The Elizabeth R. Griffin Research Foundation provides grants for research and training through its partner organizations, one of which is the American Biological Safety Association (ABSA). The ABSA web site posts a Request For Proposals in the June-July timeframe and makes resources developed through the grant available free of charge on its web site. While considerably more funding is needed, these organizations provide a good starting place for those investigators seeking to compete for grants and conduct research in animal biosafety.

References


Containment Talk

J. Paul Jennette¹, Miguel A. Grimaldo², and John R. Henneman³

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Welcome to our new column in Applied Biosafety! In this column, three of ABSA’s experts in the areas of containment equipment and facilities operations will answer questions about a variety of containment topics, including subjects such as:

- What is the “best” type of biological safety cabinet?
- Are there options for when the thing you need to contain won’t fit in a BSC?
- Is there a “correct” differential pressure for containment?
- What is the best way to control directional airflow and pressures?
- When (and where) is an airflow reversal in an A/BSL-3 facility okay?
- What is the best method for decontaminating a containment facility?
- What is the proper autoclave decontamination cycle time?
- Does my lab need an effluent decontamination system?
- How does the term “certification” relate to containment labs?

Of course, the first answer to all of these questions is “IT DEPENDS!” but the authors hope that their collective experience will help our readers with questions like these. This column will be most effective when we can answer questions from our readers. Please e-mail your questions on anything related to biocontainment facility design, operations, maintenance, and biosafety to Paul Jennette at jjpj22@cornell.edu, Co-Editor Barbara Johnson at barbara_johnson@verizon.net, or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Meet the Containment Guys

For this issue, we’ll start with a brief introduction to the authors of this column (aka, the Containment Guys):

Miguel A. Grimaldo, ME, is the Director of Institutional Biocontainment Resources at the University of Texas Medical Branch (UTMB) and the Director of the Biocontainment Engineering Division for the Galveston National Laboratory. His responsibilities include the review of all design, construction, and commissioning of high- and maximum-containment laboratories. He also ensures regulatory compliance and conducts ongoing evaluation and recertification on all critical containment features, equipment, and operations for Biosafety Level 3 (BSL-3), Animal Biosafety Level 3 (ABSL-3), and Biosafety Level 4 (BSL-4) laboratory facilities at UTMB. He also is a member of the UTMB Institutional Biosafety Committee. Miguel obtained his Masters in Engineering from the University of Louisville and Bachelor of Science degrees in Agricultural Engineering and Agricultural Economics from Texas A&M. Before coming to UTMB in 2005, Miguel worked for 13 years for the U.S. Department of Agriculture (USDA) at the U.S. Embassy in Panama, where he was involved with USDA’s Foot and Mouth Disease Prevention Program as well as the Screwworm Eradication Program. Also, Miguel has served as a Biocontainment Advisor for laboratories nationally and internationally. He currently serves as one of ABSA’s representatives to the NIH Design Requirements Manual revision committee and is also a member of the ANSI committee to develop a national standard for the verification of BSL-3 facility performance.

John R. Henneman, MS, RBP, is the Manager of the Pell ABSL-3 Laboratory for Advanced Biological Research at The Pennsylvania State University. He has held this position for about two years and also serves on the PSU Institutional Biosafety Committee. John managed the day-to-day facility operations of an ABSL-3 laboratory at Battelle, (West Jefferson, Ohio) from 1995 to 2010, and previously had over 13 years of experience in scientific research as a contractor for the National Cancer Institute. At Battelle, he oversaw facility maintenance and worked with over 200 research staff and numerous contractors to schedule and carry-out projects safely and efficiently. He also served as Chairman of their Environment, Safety, Health, and Surety Committee for 9 years. John’s expertise includes the design, building, commissioning, and safe operation of biocontainment facilities, along with the unique ability to understand research needs and equip a facility to accommodate them. He is a graduate of Mansfield University of Pennsylvania and Hood College, Frederick, Maryland, with degrees in Biology and Environmental Biology. John is an instructor for classes involving containment laboratories offered by the Eagleson Institute. He serves as one of ABSA’s representatives to the NIH Design Requirements Manual revision committee and is also a member of the ABSA task team supporting ABSA’s involvement in the ANSI committee to develop a national standard for the verification of BSL-3 facility performance.

J. Paul Jennette, MS, PE, RBP, is the Biosafety Engineer at Cornell College of Veterinary Medicine. His responsibilities include design, verification, operation, decontamination, maintenance, training, and program management related to the College’s BSL-3 and ABSL-3 facilities. He also directs the operations of the College’s medical waste management program which includes a number of BSL-3 autoclaves and a large Rotoclave for central medical
waste treatment, as well as a large alkaline hydrolysis “digester” for the disposal of animal remains. Paul is a member of the Cornell Institutional Biosafety Committee and directs the Vet College’s rabies risk management program. Before taking this position in 1999, he worked in the environmental engineering field with a specialty in industrial wastewater management. Paul is a graduate of Cornell University (BS, Civil and Environmental Engineering) and the University of Massachusetts (MS, Environmental Engineering), and is a Registered Professional Engineer as well as a Registered Biosafety Professional. As a member of ABSA’s Professional Development Team, Paul is co-coordinator for ABSA’s Principles & Practices of Biosafety class as well as an instructor for BSL-3 courses offered by ABSA and the Eagleson Institute. Paul serves as ABSA’s representative on the ANSI committee to develop a national standard for the verification of BSL-3 facility performance.

Capsule

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

**Nosocomial Transmission of Measles, Environmental Viral Hazards from Food and Water, Lethality of H5N1, and Laboratory-acquired Brucellosis in Turkey**

**Nosocomial Transmission of Measles: An Updated Review**

Bothelo-Neversa et al. (2012) report that on a global level over the past decade, improved vaccination coverage has substantially decreased the incidence and mortality of measles. In the Americas, endemic measles has been eliminated; in the Pacific region, elimination goals are within reach. However, in Southeast Asia and Africa, many countries are still at risk of missing the WHO elimination targets. In Europe, the incidence has declined too, except in Western Europe where it has increased because of suboptimally vaccinated pockets of individuals (antivaccination groups) and people with limited access to health care (for instance, the Roma ethnic group that was displaced from Eastern to Western Europe).

With the elimination of endemic measles, transmission in healthcare settings has become more relevant. Risk can be thought of as a function of likelihood and severity. On the likelihood side, a number of factors favor rapid spreading of nosocomial measles (Bothelo-Neversa et al., 2012). With a basic reproduction number of between 7.7 and 15, measles is highly contagious even before the onset of rash-es. The early signs are nonspecific and include fever, cough, and conjunctivitis, which are very similar to common cold symptoms. Hence, because of this unspecific presentation, healthcare workers in regions with low measles incidence may miss the symptoms as potentially contagious measles and, as a consequence, patients may have already transmitted the virus in waiting and admission areas. The virus can persist in aerosols for extended periods, which is ideal for transmission in crowded settings such as healthcare facilities (Bothelo-Neversa et al., 2012).

On the severity side of the risk equation, nosocomial measles is more severe than the community-acquired illness. Patients in emergency care units and triage wards, as well as those with underlying risk factors, are at a higher risk.


**Virus Hazards from Food, Water, and Other Contaminated Environments**

Rodríguez-Lázaro et al. (2012) report in a review article that numerous pathogenic viruses of human and animal sources can be transmitted by water and food, and infect people after ingestion and occasionally through skin contact. The article begins with a systematic overview of the
relevant food and environmental virus hazards and reveals by which routes these viruses are shed into the environment and reach food and drinking water.

The most important human pathogenic viruses in this context include the families of the Caliciviridae, Adenoviridae, Hepeviridae, Picornaviridae, and Reoviridae. For these, Rodríguez-Lázaro et al. (2012) review sampling methods, first-choice detection and surveillance methods, and their limitations.

The caliciviruses include norovirus and sapovirus, which are the most important viral agents for acute gastroenteritis and diarrhea in all age groups worldwide. The major route of transmission is food and water contaminated with fecal matter and vomitus, fomites, and person-to-person contact. Sapoviruses affect mostly young children.

Adenoviruses include numerous serotypes, some of which cause gastroenteritis in children by the fecal-oral route. Water-borne outbreaks have been reported after visiting swimming pools with insufficient water disinfection.

Hepatitis E virus is the sole member of the Hepeviridae and is a relevant cause of hepatitis in regions with inadequate water supply and poor hygienic conditions. Only isolated cases were reported in industrialized countries.

Hepatitis A virus is the most prominent representative of the Picornaviridae in this context. Infections have declined substantially in countries with effective immunization programs and improved sewage treatment and hygiene practices, which have severely curtailed the fecal-oral route of transmission.

The Reoviridae family includes rotavirus and astrovirus, which are water-borne pathogens affecting mostly children, and prominent agents of gastroenteritis.

The above-mentioned viruses can be of zoonotic or human origin, and disease is associated with the consumption of contaminated water and food of animal origin or after manipulation by infected humans. Contaminated animal feces can contaminate crops if used directly as manure or indirectly after reaching surface water that is used for irrigation. Contaminated human feces can also reach surface water used for irrigation of fruits and vegetables. Rodríguez-Lázaro et al. (2012) report that most pathogenic viruses emerging in human populations are of animal origin. Once an outbreak happens in a community, human-human transmission, contamination of food by food handlers, and fomite-human transmission become relevant too. After an outbreak, healthcare workers are the first who face an occupational exposure, and nosocomial-related infections can spread quickly.

Good sampling strategies are important for the early and accurate identification of environmental virus hazards and allow for deploying rapid and successful countermeasures, but they are not cheap. Cost-benefit analyses are not available. The authors (Rodríguez-Lázaro et al., 2012) cite various international bodies, such as the International Organization for Standardization (ISO), the European Committee for the Normalization (CEN), the European Food Safety Authority (EFSA), and national bodies, such as the U.S. Department of Health and Human Services (USDHHS), that have defined principles and/or standards for the sampling of food and water, and mention that the World Health Organization (WHO) and the European Centre for Disease Prevention and Control (ECDC) have devoted considerable energy to developing integrated surveillance networks.

The last section of the review (Rodríguez-Lázaro et al., 2012) focuses on detection methods, as well as on evaluation and interpretation of test results. A short paragraph is devoted to laboratory safety. The large variety and complexity of samples and the possible heterogeneous distribution of a small number of viruses in the sample render detection not always easy. In addition, the presence of test inhibitors may interfere with the detection methods. Often, to isolate virus from environmental samples, a concentration step is required before a test can be done. The available detection tests include cell culturing (plaque assay, TCDL₅₀ [tissue culture infective dose assay]), molecular methods (PCR [polymerase chain reaction], real-time PCR, NASBA [nucleic acid sequence-based amplification]), as well as immunological methods (EIA [tissue culture infective dose assay], RIA [radioimmunoassay], and ELISA [enzyme-linked immunosorbent assay]). After detection, an identification or characterization test is required (sequencing, multiplex PCR, immunostaining, etc.). The authors (Rodríguez-Lázaro et al., 2012) discuss the strengths and weaknesses of the tests. A test method may be useful for one type of virus, but not for another.

The topic of so-called index viruses is also presented. Classical indicators such as fecal coliforms are still in use, but recently their adequacy has been questioned. To this day, available data are not yet sufficient to use index viruses as a more accurate or sensitive tool to monitor the quality of water and food.


Is H5N1 Really Highly Lethal?

Toner and Adalja (2012) stress that the answer to this question is central in the current debate over research on genetically modified H5N1 influenza viruses. The authors mention that not all H5N1 viruses are equal; some, which are so-called low pathogenic H5N1, are not closely related to the viruses of concern. The lineage of the original highly pathogenic avian influenza strain that has evolved over the last 15 years includes several descendent strains and sub-strains, with different death rates. As of January 2012, WHO has registered 583 people with an H5N1 infection, from which 344 have died. The fatality rate of WHO-confirmed cases is 59%. The figures are not challenged, but the question remains as to whether all human infections have resulted in symptoms and how many have gone un-
noted. The authors (Toner & Adalja, 2012) identified 22 seroepidemiological studies; the majority of these studies found no or little evidence of subclinical H5N1 infections. Four studies had to be excluded for methodological reasons. Of the remaining 18 studies, in 11,477 persons only 144 were found to be seropositive (1.25%). The authors note that many of the 18 studies suffer from inadequate design and methods and conclude that it is impossible to draw a final conclusion on what the prevalence of asymptomatic seroconverters means in terms of fatality rate. For instance, an irrelevant seroconversion could have happened after an exposure to contaminated water. Toner and Adalja (2012) confirm that people who are infected become seriously ill and a large proportion die. In response to this article, Zelicoff (2012) writes in a letter to the editor that “morbidity from novel influenza strains does not equate with an impending pandemic, let alone one with high mortality.” Zelicoff (2012) suggests that “it would appear likely that a systematic, prospective cohort study is in order to adequately capture the frequency of asymptomatic infection.”


Laboratory-acquired Brucellosis in Turkey

Sayin-Kutlu et al. (2012) report about a multicenter survey conducted in 38 hospitals in 17 of the 81 provinces in Turkey. Thirty-eight of the 667 interviewed laboratory workers had experienced a lab-acquired brucellosis (LAB, 5.8%). Statistical analysis revealed the three most important factors that reduce the likelihood of an LAB are using a biosafety cabinet class II (odds ratio 0.27, P value 0.009), full adherence to glove use (odds ratio 0.27, P value 0.004), and longer experience as a lab worker (odds ratio 0.86, P value <0.001). Performing bacterial isolation, being a staff member, and being male increased the odds of an LAB significantly (5.12, 3.21, and 2.14, respectively). Brucellosis is still endemic in Mediterranean and Middle Eastern countries, including Turkey. In many of these countries, laboratory facilities and equipment are not appropriate. In the Turkish study (Sayin-Kutlu et al., 2012), the interviewees diagnosed with LAB named inadequate facilities such as lack of biosafety cabinets and tools (79%), lack of knowledge about the mode of transmission (52%), and ignoring the risk of infection (64%) as the cause of LAB.


Beyond Traditional Biosafety

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Biosafety professionals have ever-expanding roles at their institutions. In this Beyond Traditional Biosafety column, we focus on topics that may fall outside the scope of the traditional biosafety role, but where the expertise of the biosafety professional may be called upon to provide a valuable contribution to his or her institution. Please e-mail any comments or suggestions to Ted Myatt, tedmyatt@gmail.com, Co-Editor Barbara Johnson at barbara_johnson@verizon.net, or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Construction in Healthcare Facilities—Risks from Opportunistic Fungi and Control Solutions

Construction, demolition, and renovation activities are common occurrences in a healthcare facility. Hospitals are constantly seeking to better utilize current space, as well as to expand to accommodate growing needs. Potentially, construction work in hospitals can place patients, staff, workers, and visitors at risk for serious health issues. These include risks from inhalation exposure to opportunistic fungi, the focus of this article.

Airborne dust and other debris generated during construction work can contain opportunistic fungi that are generally harmless to the average person, but can cause serious infections in severely immunocompromised patient populations. The primary infection control concern during hospital construction is exposure to fungal elements that can cause a group of invasive fungal respiratory diseases. One such disease, aspergillosis (caused by Aspergillus spp., most commonly A. fumigatus, A. flavus, and A. terreus), can affect compromised patients such as those undergoing high-dose chemotherapy for hematological malignancies with or without stem cell or bone marrow transplants, and those with auto-immune disease, solid organ transplants, and immunosuppressive diseases such as AIDS (Kauffman, 2010; Maschmeyer, 2007; Muller, 2002; Yeghen, 2000). Aspergillosis can take several forms. These include nonin-
vasive forms such as in the formation of a fungus ball in aspergillosis, hypersensitivity disease in conditions such as acute bronchopulmonary aspergillosis, and invasive disease such as that involving the lungs (invasive pulmonary aspergillosis), skin (invasive cutaneous aspergillosis), or multiple organ systems (disseminated aspergillosis).

Invasive pulmonary aspergillosis (IPA) is the form most often associated with construction activities. The mortality rate for IPA ranges from 58% to nearly 90% in bone marrow transplant recipients undergoing high-dose chemotherapy treatment (Lin, 2001). In addition to aspergillosis, airborne contaminants created during construction activities can create acute irritation in healthy patients, staff, workers, and visitors.

To control airborne dust and debris from travelling outside the construction site, the worksite should be isolated from the surrounding occupied areas. Members of the construction team, as well as Infection Control and Project Management personnel, should work together to ensure that construction work is segregated from hospital populations. Guidance for managing risks from construction in hospitals is provided by two groups—The Facility Guidelines Institute (FGI) and The Joint Commission (TJC) (FGI, 2010; TJC, 2009). The FGI has published several recommendations that hospitals should implement before and during construction activities to ensure that high-risk patients are protected. These include physically isolating the worksite from surrounding areas, isolating the mechanical systems, and maintaining the worksite at a negative pressure relative to surrounding occupied areas. TJC subsequently adopted the FGI guidelines for controlling the potential impacts from construction, as described in Standard EC.02.06.05 (TJC, 2009). This standard outlines the hospital’s need to manage risk associated with construction through design and complete a pre-construction risk assessment (PCRA). An important element of the PCRA is the Infection Control Risk Assessment (ICRA), which focuses on the impact construction can have on the health of the patients, staff, and visitors. The PCRA should consider air quality, infection control, noise, vibration, and utility interruptions that may occur during construction work.

An effective method to isolate the construction site is to maintain a worksite that is at negative pressure to surrounding occupied spaces for the duration of the project. To do this, negative-air machines are typically used to draw in air from the construction zone which is then filtered through high efficiency particulate air (HEPA) filters in the machine and exhausted to the outdoors. To ensure that pressure is kept negative, regular pressure measurements are collected. On critical projects occurring near immunocompromised patient populations, continuous pressure monitors may be installed at the project entrance. Also, potential impacts to adjacent spaces need to be evaluated prior to using negative-air machines. For example, an isolation room or other area with critical airflow patterns and pressure relationship that is adjacent to the construction site requires that the airflow patterns be evaluated to ensure that the existing pressure relationship is never compromised.

In addition to adjusting the pressure in the work space, several other control methods need to be implemented to prevent the potential transfer of opportunistic fungi to surrounding areas. These include the following:

- Construct barriers that physically separate the construction work zone from surrounding areas to prevent the transfer of dust and other airborne contaminants. The barrier materials should be fire retardant and sealed tightly.
- Isolate or shutdown existing heating, ventilating, and air-conditioning (HVAC) systems serving the work area for the duration of the project.
- Fully cover all containers used for the transport of tools, materials, or debris and ensure that they are visibly free from dust accumulation (including the wheels).
- Use designated transport routes and elevators (if necessary) to remove materials from the worksite, and clean these routes daily.
- Clean the worksite regularly (at a minimum at the end of the work shift) using damp-wiping and wet-sweeping methods to control dust.
- Install sticky mats at each entrance/exit of the containment area and change these mats at the end of each work day, or more frequently if necessary.
- Sequence material delivery and debris removal to occur at times when outpatient traffic is low and/or when patients are not expected to be within the corridor.
- After completion of the construction work, and prior to containment disassembly, thoroughly clean all dust and debris from the work zone by vacuuming with a HEPA-filtered vacuum and wiping with a damp cloth.

A critical component of the overall risk management plan to maintain the construction site is to have a process to verify controls that are in place. This includes regular verification of the air pressure, cleaning effectiveness, and containment setup. Additionally, biological air monitoring may also need to be considered. Although no standards for fungi concentrations in air exist, airborne particle counts and cultivatable fungi from air sampling are often used to evaluate the effectiveness of isolation measures, as well as to qualify a space following construction. Studies have shown that nosocomial infections appear when Aspergillus spp. levels range from 0.9 - 2.2 colony forming units per cubic meter of air (cfu/m³) (Sehulster & Chinn, 2003). Many experts use <1.0 cfu/m³ as an acceptable criterion for areas of the hospital where there are immunocompromised patients (Sehulster & Chinn, 2003).

Isolating construction work from surrounding areas has been shown to decrease the incidence of infectious diseases. One study found that implementation of an infection control strategy during construction significantly reduced the incident density (ID) of nosocomial aspergillosis from 9.88 per 1,000 days at risk to 2.91 per 1,000 days at risk, comparable to the preconstruction baseline (3.18 per 1,000 days at risk) (Loo, 1996).

Many large teaching hospitals have already undergone
construction risk assessments to help in identification and reduction of any risk due to infection of immunocompromised patient populations. Important lessons learned when working to prevent the potential transfer of opportunistic fungi to surrounding areas include:

- Involve the Infection Control Team early and often within the PCRA and ICRA processes.
- Ensure that the hospital team possesses the correct skill sets and competencies to evaluate and mitigate risks due to construction, including industrial hygiene, engineering and building systems, site isolation and pressurization, and epidemiology.
- Train contractors to properly isolate construction areas from sensitive patient populations; hold contractors accountable for proper and safe work practices.
- Establish an effective epidemiological surveillance program for at-risk patient populations, monitor for invasive fungal infections as well as other nosocomial infections, and follow-up on any infection trends potentially associated with periods during construction.
- If air sampling (i.e., relative pressure, airborne dust, and mold) is conducted to evaluate the effectiveness of mitigation measures, ensure that the criteria for when to sample, what to sample, and the metric for acceptance are established prior to conducting the air sampling.
- Verify that the newly constructed area meets acceptable criteria before patients are moved into high-risk areas; this includes criteria for work practices, cleaning procedures, and mechanical and building systems.

Biosafety professionals who are employed at institutions with a hospital or are affiliated with a hospital should be aware of the potential biohazard risks associated with construction in healthcare facilities. Those professionals who understand the importance of site isolation and how to implement and verify containment will be better prepared to assist Infection Control professionals and other healthcare team members to protect the health of patients, staff, workers, and visitors.

References


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