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Guest Editorial

The Virtual Biosecurity Center: Enhanced Global Security Through Superior Communication
Kelsey A. Gregg and Sudha Siva ................................................................. 204

Articles

Thermal Inactivation of Avian Viral and Bacterial Pathogens in an Effluent Treatment System
Within a Biosafety Level 2 and 3 Enhanced Facility
Revis Chmielewski, Michael Day, Stephen Spatz, Qingzhong Yu, Richard Gast, Laslo Zsak, and David Swayne ......................................................... 206

Laboratory Safety for Oncogene-Containing Retroviral Vectors
John T. Gray .................................................................................................. 218

International Biosafety and Biosecurity Challenges: Suggestions for Developing Sustainable
Capacity in Low-resource Countries
Robert A. Heckert, J. Craig Reed, Felix K. Gmuender, Maureen Ellis, and Willy Tonui ................................................................. 223

Enhancement of the Mentored Training Program for Investigative Staff at the University of
Pittsburgh Regional Biocontainment Laboratory
Lesley C. Homer, Amy L. Hartman, Dennis T. Heflin, Anita M. Trichel, Douglas S. Reed, and Kelly Stefano Cole ......................................................... 231

Biosafety Competencies in Developing Countries: The Role of Universities
Ana Sanchez, Jose A. Gabrie, Ada A. Zelaya, Lourdes Enriquez, Maritza Canales, and Sean G. Kaufman ................................................................. 240

Special Features

Ask the Experts—Some Researchers Claim That Their Laboratories Must Be Under Positive
Pressure to Keep Unwanted Materials from Entering and Contaminating Their Work. Is It
Appropriate to Have Positively Pressurized Laboratories for Any Reason?
John H. Keene ............................................................................................... 253

Animal Bytes—Small Animal Containment Caging
Barbara Johnson ............................................................................................ 254

Capsule—Update on Vaccine-derived Polioviruses, Evaluation of RT-PCR for Differentiation of
Mycobacterium tuberculosis Complex, Transduction of Human Cells with Polymer-complexed
Ectropic Lentivirus, and Transmission Dynamics of Pneumonic Plague
Felix K. Gmuender ......................................................................................... 256
### About the Cover

Work conducted in Biosafety Level 3 and Animal Biosafety Level 3 laboratories typically involves pathogens that are transmissible via the aerosol route that are capable of causing disease with moderate to severe illness, and can include work with species of animals that pose additional hazards to laboratory personnel. Mitigating the risk of exposure and injury in these laboratories is of paramount importance and employs the combined use of best practices, primary containment, personnel protective equipment (PPE), safety devices and equipment, and engineering controls. But what type of training is needed, and how does a supervisor know when a person is competent to work in the lab independently? Training has numerous components that include general safety practices and safety theory which progresses to task specific safety practices and Standard Operating Procedures (SOPs), entry and exit procedures, room and suite specific procedures, use of PPE and equipment, animal handling, incident and accident reporting, etc. These components include training under normal operating conditions, during emergencies, systems failures and in the event of a suspect or known exposure. Training is often conducted in a layered approach to include a review of manuals and SOPs, classroom training, hands-on training with a skilled and knowledgeable mentor that may start with less hazardous organisms, progress to a “watch one, do one” approach, and culminate in demonstration of competency. The University of Pittsburg Regional Biocontainment Laboratory has implemented a thorough training and mentorship program. To learn more, see the article entitled “Enhancement of the Mentored Training Program for Investigative Staff at the University of Pittsburgh Regional Biocontainment Laboratory” by Lesley C. Homer et al. on pages 231-239. The image is courtesy of University of Pittsburgh (© 2011, University of Pittsburgh, used with permission) and shows a trained researcher wearing a powered air purifying respirator while working in the biological safety cabinet inside the containment laboratory.

### Training Announcements

#### Principles & Practices of Biosafety (PPB)

The Principles & Practices of Biosafety 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. To register for the PPB at the Embassy Suites San Diego Bay-Downtown in San Diego, California from February 26 through March 2, 2012, go to www.absa.org/eduppb.html

#### Webinars

A “Call for Webinars” is posted on the ABSA web site at www.xcdsystem.com/absa2011/webinar.cfm

#### ABSA/ERGRF Leadership Institute

The American Biological Safety Association and the Elizabeth R. Griffin Research Foundation are partnering to offer the ABSA/ERGRF Leadership Institute. The Leadership Institute is an experience designed for biosafety professionals and other leaders who may support the biosafety profession. Participants have the opportunity to challenge themselves and biosafety experts through interactive small group exercises and discussions. The Leadership Institute provides many professionals with the opportunity to explore solutions for common problems. Together, the small group exercises and discussions, fosters leadership skills and abilities which are increasingly needed for today’s biosafety practitioner. This course will be held on April 22-25 at the Embassy Suites Lake Buena Vista in Orlando, Florida. Additional information will be posted soon to the ABSA web site (www.absa.org).

#### Review Course

The Review Course is a 2-day instructor-led course that provides a comprehensive overview of the essential elements of biological safety as prescribed in the NRCM Specialist Microbiologist Task List for Biological Safety Microbiology. This course will be held on April 26-27 at the Embassy Suites Lake Buena Vista in Orlando, Florida. Additional information will be posted soon to the ABSA web site (www.absa.org).
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Deadline Dates

January 20 for Spring issue
July 20 for Fall issue
April 20 for Summer issue
October 15 for Winter issue

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The Virtual Biosecurity Center: Enhanced Global Security Through Superior Communication

Kelsey A. Gregg* and Sudha Siva
Federation of American Scientists, Washington, DC

Biosecurity and biosafety associations are scattered around the world and across various types of organizations, including academia, public health, government, non-governmental, law enforcement, and industry. Keeping up with recent news, events, reports, and projects in the biosafety and biosecurity community has required considerable time and effort each day searching numerous sites and postings. This communications barrier, in addition to a lack of funding, has hindered progress within our community. As a leader in biosecurity outreach and education, the Federation of American Scientists (FAS) is committed to addressing these barriers and to help make the world safer, healthier, and more secure by initiating the Virtual Biosecurity Center (VBC).

The VBC web site was launched on February 24, 2011, and serves as the “one stop shop” for biosecurity news and information worldwide. This web site hosts a comprehensive listing of global biosecurity and biosafety conferences, events, educational resources, compilation of biosecurity codes of conduct, organizations, and governmental agencies in addition to a library, videos, and career section which are updated continuously with the most current information. The VBC offers a platform for experts in the community to showcase their work and opinions through VBC Op-Eds and the Global Forum on Biorisks, a collaborative online forum for informing policy and empowering partnerships among professional biosecurity communities. To truly be a global initiative, the VBC is translated into more than 50 languages, including the six official United Nations languages, allowing almost everyone to share news, events, and information, collaborate, research best practices, and access the reference materials they need in real time to help counter global biological threats.

In addition to being the online communications hub for biosecurity, the VBC is reaching out to the life sciences and security communities through funded graduate fellowships and international conferences. Our first graduate fellow evaluated the impact of funding, collaboration, and geographic location on biosecurity programs worldwide. The fellow also contributed to an agriculture education module and assisted with the VBC’s first international conference, “Taking Biosecurity Networks to the Next Level.” This conference brought together over 75 scientists and policymakers to investigate the role of web-based networks in global biosecurity. As the first conference of its kind in the biosecurity community, we wanted to do things a little differently. Our thought-provoking presenters, stimulating break-out sessions, audience response system, and live interactive webcast kept the conference fully engaging, both to those attending in person and virtually.

Another way the VBC supports the global biosafety and biosecurity community is through directly funding projects. Through our financial support, Biosafety Biosecurity International and the International Centre Diarrhoeal Disease Research, Bangladesh developed a local, sustainable, and multi-pronged approach to strengthen biosecurity and biosafety in Bangladesh. As a result of its laboratory analysis, workshops, and outreach, Bangladesh is well underway to establishing its first biosafety and biosecurity organization. In addition to this biosafety project, the VBC funded work on the documentary “Making Anthrax” by Cornell University. This project explores the social and ethical dimensions of biological weapons research and development, featuring interviews with former Soviet bioweapons scientists. This work should be available to the public in 2012 and will be featured on the VBC web site.

The VBC and its initiatives were made possible through the collaboration of FAS and an international network of leaders in biosecurity, biosafety, science policy, and the life sciences. Our Board of Advisors includes individuals from prominent organizations such as the American Association for the Advancement of Science, the National Academy of Sciences, and the Organisation for Economic Co-operation and Development. The VBC has also partnered with 31 other prominent organizations that are committed to our goals and to sharing their activities and educational materials through the VBC. These participating organizations include many biosafety organizations, such as the African Biological Safety Organization, the Moroccan Biosafety Association, the International Federation of Biosafety Associations, the National Biosafety Association in Brazil, and the American Biological Safety Association.

The VBC is supporting high-impact, long-lasting, novel projects in biosafety and biosecurity, fostering lasting
collaborations, educating the public and policymakers through easily accessible and consumable information, and modernizing communication within the biosafety and biosecurity communities. The VBC can help secure the world outside of the biosafety and biosecurity communities by serving as a collaboration and communications model for other policy areas. This can increase efficiency and reduce wasted time and duplicated efforts, allowing other policy areas to focus on solving their issues, instead of simply trying to stay up-to-date in their fields. The VBC is looking forward to further engagement with the biosafety community to advance our ultimate goals of making the world safer, healthier, and more secure.

For more information about the VBC, or to view the first international VBC conference, please visit our website at www.virtualbiosecuritycenter.org.

Acknowledgments

The Virtual Biosecurity Center (VBC) was developed with a grant from the U.S. Government, Office of the Director of National Intelligence, and the Carnegie Corporation of New York. *Correspondence should be addressed to Kelsey A. Gregg at kgregg@fas.org.

Calendar of Events

**February 26—March 2, 2012**
ABSA Principles & Practices of Biosafety (PPB)
Embassy Suites San Diego Bay—Downtown, San Diego, California
*Contact:* Phone: 1-866-425-1385; E-mail: info@absa.org; www.absa.org

**October 18-24, 2012**
ABSA 55th Annual Biological Safety Conference
Hilton Bonnet Creek Hotel, Orlando, Florida
*Contact:* Phone: 1-866-425-1385; E-mail: info@absa.org; www.absa.org

**November 4-8, 2012**
American Association for Laboratory Animal Science (AALAS) 63rd National Meeting
Minneapolis, Minnesota
*Contact:* http://nationalmeeting.aalas.org/future_sites.asp
Thermal Inactivation of Avian Viral and Bacterial Pathogens in an Effluent Treatment System Within a Biosafety Level 2 and 3 Enhanced Facility

Revis Chmielewski, Michael Day, Stephen Spatz, Qingzhong Yu, Richard Gast, Laslo Zsak, and David Swayne*

United States Department of Agriculture, Athens, Georgia

Abstract

Avian influenza (AI) virus, avian paramyxovirus Type 1 (APMV-1 or NDV), reovirus, rotavirus, turkey astrovirus (TAvSt), avian metapneumovirus (aMPV), Marek’s disease virus (MDV-1), avian parvovirus (ChPV), and Salmonella enterica serovar Enteritidis are significant biosafety level 2 (BSL-2) or biosafety level 3 enhanced (BSL-3E) pathogens of poultry that are studied in veterinary medical research laboratories worldwide. The purpose of this study was to determine the effectiveness of a moderate temperature, effluent decontamination system (EDS) to inactivate avian pathogens. First, the thermal inactivation processes for AI virus, APMV-1, reovirus, and rotaviruses were determined in phosphate-buffered saline (PBS) using in vitro assays from which thermal death rates (D) and changes in heat resistance (zD) of the AI virus and APMV-1 were determined at various time/temperature parameters. The PBS validation process demonstrated that 6 log10 reduction was achieved following heating at 82.2°C within 30 seconds for AI virus and APMV-1, 1.8 minutes for astrovirus, while reoviruses and rotaviruses were destroyed within 3 minutes. Second, to determine whether pathogens were adequately inactivated in a moderate temperature EDS system, vials containing avian viruses (5.1-11.1 log10 TCID, EID50/ml, or infectious particle/ml) and bacteria (9.1 log10 cfu/ml) were placed in the effluent tank and exposed to a standard cycle of 82.2°C for 6 hours. The EDS process totally inactivated enveloped and non-enveloped viruses with complete inactivation of greater than 5.1-11.1 log10 TCID/ml of specific pathogens. The data from the inactivation models and the EDS validation test showed that the 6 log10 reduction required for a sanitary assurance level of effluent was achieved with a significant margin of safety.

Introduction

Liquid effluent waste from Biosafety Level 2 and 3 Enhanced laboratories (BSL-2E and BSL-3E) and animal facilities (ABSL-2E and ABSL-3E) must be decontaminated before discharge into the public sewer system. The Clean Water Act (CWA) of 1972 sections 301(a,b), 304(b), 304(g), 304(m), and 307(b) and the secondary treatment regulation prescribe water quality discharges of pollutant in the wastewater (Anonymous, 1972; Anonymous, 1981). The intent of the effluent guidelines and pretreatment standards is reduction of chemical, physical, and biological contaminants discharged to surface water. One measure to prevent biological contaminants in a water source is pretreatment of wastewater through indirect steam heating prior to discharge into the surface water or public sewer system. Most biohazard inactivation efficacy tests are based on the inactivation of bacterial spores. Microbes and transmissible agents have varying degrees of tolerance to heat; the most thermally resistant are prions, followed by (in decreasing order of thermal resistance) bacterial spores, mycobacteria, nonlipid or naked viruses, fungi, vegetative bacteria, and lipid encapsulated viruses (CDC, 2007). Historically, the validation process for effluent discharge from an effluent decontamination system (EDS) has used a spore test to ensure sterility at an assurance level (SAL) where a 6 log10 reduction is achieved by the steam inactivation process for those thermal-resistant microbes (CDC, 2007). Therefore, the focus of the decontamination process is to inactivate not only pathogens used within the specific laboratory, but also to prevent a theoretical most severe case scenario.

Heat inactivation studies have not been reported for enveloped and non-enveloped avian viruses and vegetative bacteria for purposes of effluent decontamination. However, heat inactivation studies at 60°C-70°C for enveloped avian influenza and Newcastle disease viruses have shown that the time required to reduce the viruses by 6 logs in homogenized whole eggs and chicken meat was less than 2 minutes and 4 minutes, respectively (Swayne et al., 2004; Thomas et al., 2008).

The purpose of this study was to determine inactivation of enveloped and non-enveloped avian viruses and a vegetative bacterium in an operational indirect steam effluent decontamination system (EDS). We also developed predictive models to validate the inactivation
for low-pathogenicity notifiable avian influenza virus (LPNAIV), high-pathogenicity avian influenza virus (HPAIV), and virulent (vNDV) and lentogenic Newcastle disease viruses (INDV), at various time/temperature parameters. Linear or non-linear regression models were used to obtain thermal inactivation rates and changes in thermal resistance (Dₐ and zD) in a phosphate buffer system.

**Materials and Methods**

**Thermal Inactivation Modeling of Pathogens**

**Avian Influenza and Newcastle Disease Viruses.** Viruses were diluted with phosphate-buffered saline to achieve a median embryo infectious dose of 6-8 log₁₀ (EID₅₀)/ml (Table 1). Ninety microliter (µl) aliquots of the inoculated PBS were dispensed into 200 µl thin-walled polypropylene tubes. Samples were equilibrated at 25°C for 2 minutes, then heated to the desired temperature. Upon reaching the desired temperature, samples at the zero time point were removed and the timing commenced. Samples were heated at 53°C, 55°C, 57°C, 59°C, 61°C, and/or 63°C for 0, 1, 2, 3, 4, 6, 8, 12, 15, and 20 minutes in a PCR thermocycler block (GeneAmp® PCR System 9700, Perkin Elmer, Boston, MA), and then immediately cooled to 4°C in a cooling block (Diversified Biotech, Dedham, MA). For some sampling times, triplicate samples were pooled. The purpose of the pooling was to obtain sufficient volume (270 µl) to titer the undiluted sample (10⁶) and increase the detection limit to 1 log₁₀. For viral assay titration, samples were serially diluted (10⁰ to 10⁷) in media (MEM with antibiotics) for inoculation into cell cultures. Each experiment was replicated in triplicate.

**Reoviruses and Rotaviruses.** Ninety microliter aliquots of inoculated samples of reovirus (Reo/TX/98) and rotavirus (Ro/1145/08) (6 log₁₀/ml EID₅₀) were dispensed into 200 µl thin-walled thermocycler polypropylene tubes (Table 1). Samples were equilibrated at 25°C for 2 minutes then heated to the desired temperature. Samples were exposed to an anisotermic inactivation by linear ramping of temperatures from 30°C to 82.2°C within 150 seconds in a PCR thermocycler block. Upon reaching 82.2°C, samples were removed and immediately cooled to 4°C in a cooling block.

**Tissue Culture Assay.** Monolayer chicken embryo

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### Table 1
The names and strains of enveloped and non-enveloped avian viruses used in the validation process of the effluent decontamination system.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Genetic Characteristics</th>
<th>Acronym</th>
<th>Strain</th>
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<tr>
<td><strong>Enveloped Viruses</strong></td>
<td></td>
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<tr>
<td>Avian metapneumovirus</td>
<td>RNA, ss-</td>
<td>aMPV-CO</td>
<td>aMPV/Colorado/96/C</td>
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<tr>
<td>Marek’s disease virus</td>
<td>DNA, ds</td>
<td>MDV/CV1988</td>
<td>Gallid herpesvirus 2 vaccine strain CV1988</td>
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<tr>
<td>Virulent Newcastle</td>
<td>RNA, ss-</td>
<td>vNDV/CA/2002</td>
<td>Chicken/United States (CA)/212676/2002</td>
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<tr>
<td>High pathogenic Avian Influenza virus</td>
<td>RNA, ss-</td>
<td>HPAI/PA/83</td>
<td>A/chicken/Pennsylvania/1370/83 (H5N2)</td>
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<tr>
<td>Low pathogenic notifiable avian influenza virus</td>
<td>RNA, ss-</td>
<td>LPNAI/NY/94</td>
<td>A/chicken/New York/13142-5/94 (H7N2)</td>
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<tr>
<td><strong>Non-enveloped Viruses</strong></td>
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<td>RNA, ds</td>
<td>Reo/TX/98</td>
<td>Turkey/Texas/SEP/98</td>
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<td>Reovirus strain</td>
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<td>Reo/TX/99</td>
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<td><strong>Vegetative Bacteria</strong></td>
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<tr>
<td>Salmonella enteritidis</td>
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<td>Salmonella enterica serov Enteritidis, phage type 13a, accession # 19299-52-1</td>
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¹ 6 hours, 82.2°C
fibroblast (CEF) cells were used to titer iNDV and vNDV, and Madin-Darby Canine Kidney (MDCK) cells were used to titer LPNAIV and HPAIV in 96-well microtiter plates. The monolayers were washed three times with PBS, inoculated with viruses, and incubated for 1 hour. Following incubation, media supplement with trypsin was added to the inoculated monolayers and incubated at 37°C under 5% CO₂ humidified atmosphere for 4 days. The viruses were titered using standard hemagglutination assays. Briefly, 50 µl aliquot of supernatant from the inoculated cells were transferred into a microtiter plate, serially diluted (1:2), then overlaid with 0.5% red blood cells (RBC). After 30 minutes of incubation, the presence or absence of hemagglutination was recorded. Virus titers were determined by calculation of TCID₅₀ (Reed et al., 1938).

**Statistical Analysis.** Inactivation curves were generated by plotting the log value of the infectious dose of the virus (TCID₅₀) as a function of the heating times at specific temperatures using Microsoft Excel 2003®. The statistical distribution of the data was examined to determine whether the model was linear or non-linear. Linear, exponential, and polynomial regression was fit to the curves. The D₅₀ or time (minutes) to inactivate the viral load by 90% was calculated from the inverse value of the slope (1/slope) of the regression plot. The z₀D value is the temperature required to change the D₅₀ by 1 log or 10-fold and was determined by computing the regression of the mean log D₅₀ versus heating temperatures. z₀D values were the absolute values of the inverse slope.

**Effluent Decontamination System Validation**

**Effluent Decontamination System (EDS) Validation Operation for the Inactivation of Avian Pathogens.** The EDS includes four non-pressurized, large, cold steel tanks that thermally treat and cool the liquid waste prior to release into the municipal sewage system (Figure 1). Each tank has a total volume of 15,142 L (4,000 gallons) with a working capacity for each cycle of approximately 12,113 L (3,200 gallons). The tanks were cylindrically shaped with dimensions of 6 meters long, 2 meters high (20’ x 6’), and surrounded by 5 centimeters of fiberglass insulation encased in plastic. Each tank contained a 4.5 m (15’)-long steam bundle with a 6.4 cm (2.5”) diameter used for heating the effluent. The steam bundle ran about three-fourths the length of the tank and was placed so that the bottom of the steam bundle was 25 cm (9.8”) from the bottom of the tank. The tank cycle included fill, warm-up, thermal maintenance, and cool off/purge. The EDS tanks contained a low-pressure (5 psi) air supply system within each tank to allow slow mixing of liquid to ensure limited temperature stratification. The EDS was non-pressurized and used a moderate temperature (minimum of 180°F [82.2°C]) for a minimum of 6 hours along with the low air pressure mixing system to ensure that the effluents were heated evenly, thus ensuring that all viral and bacterial pathogens used in the facilities would be inactivated.

**Inactivation Procedure for BSL-2 Viruses in EDS Tank.** Two milliliters (ml) of virus-containing liquid were

---

**Figure 1**

Depiction of EDS cook tank as modified in 2002. Actual schematics are not available.
added to each of four 4 ml internally threaded cryogenic vials (Corning #430491, Lowell, MA). All vials were sealed in 5-ply polyethylene plastic pouches using a vacuum packaging system (FoodSaver, Tilia International, Inc., Rye, NY). Each vial was considered as one replication for a total of three replicates for each EDS treatment plus one control per virus. Sealed vials containing titrated cultures of different viruses and bacteria were submerged into an EDS tank to evaluate survivability. Three replicates of each virus type were immersed into the EDS system, while one replicate of each virus was maintained at -70°C as a positive control. For the bacterial samples, there were four replicates for EDS treatment and four controls.

Inactivation Procedure for BSL-3E Viruses in EDS Tank. Vials containing highly pathogenic viruses currently held in BSL-3E labs were decontaminated by direct immersion into phenolic disinfectant (One Stroke Environ®, Steris Inc., Mentor, OH), placed in a plastic bag, sealed, and directly transferred to the BSL-2E laboratory where they were vacuum-sealed within a biological safety cabinet. The vacuum-sealed vials were directly transported to the EDS room in a sealed plastic bag and were immersed as described below. The entire procedure of removal of the viruses from BSL-3E, vacuum-sealing, and transfer to the EDS tank was conducted without interruption so that the BSL-3E viruses were not stored for any length of time at BSL-2E. All virus transfers between buildings were in compliance with established Southeast Poultry Research Laboratory Guidelines (SEPRL, 2009).

Tank Immersion Procedure. In this EDS validation study, there were two heating cycles. The first cycle was the inactivation of any pathogens in the effluent before opening the tank and adding the vials containing virus into the EDS. The effluent was exposed to one heat decontamination cycle, then allowed to cool to 60°C. The second cycle was the evaluation of the thermal inactivation of avian viruses within the vials. Individually sealed cryovials containing live viruses or bacteria were inserted into an expandable mesh sleeve (Halar®, Solvay Solexis, Inc., Houston, TX). The sleeve was closed and a weight was added to the bottom of the mesh sleeve to maintain the sample vials vertically at the approximate center of the tank during the thermal inactivation process. A primary barrier was set up around the tank port prior to sampling. Briefly, a plastic (CPVC) frame covering the sample port wrapped with a large 60" x 60" four-ply plastic bag was attached at the base of the sample port with duct tape. Prior to unsealing the sample port, a weighted sleeve containing the sample vials was attached to a new sample port cap with stainless steel chains. The samples along with a spray bottle of disinfectant (5.25% sodium hypochlorite, Garden Scientific, Van Nuys, CA) were placed inside the primary containment barrier (Figure 2). The unsealing of the sample port and the loading and unloading of the samples into the EDS tank were operated from outside the primary barrier. After the samples were added and the port was sealed, the tanks were cycled through a second standard decontamination cycle at 82.2°C for a minimum of 6 hours at 5 psi in the bubble/mixing system. To remove the samples, the process using the primary barrier was

**Figure 2**

A. Sealed sample vial; B. EDS tank; C. Plastic frame, disinfectant bottle, Halar® mesh sleeve and metal cap on top of EDS tank port; and D. EDS tank with attached plastic bag barrier.
performed in reverse. The sample port cap with sleeve and weight was removed from the tank and the original cap was placed on the sample port. The plastic bag containing samples was removed from the tank. All contents in the primary barrier (the cap, sleeve and weight, sample bag, the disinfectant bottle, and the inside of the plastic bag barrier) were disinfected, sealed in a plastic bag, and taken directly to the BSL-2E laboratory (Figure 2). The vials containing the highly pathogenic viruses were immediately chilled, placed in a secondary container, and taken directly to the BSL-3E laboratory for processing.

**Temperature Validation.** Thermocouples were used to monitor the temperature of the effluent in the EDS tank. The thermocouples were validated previously (ENV, Inc., New York, NY) and were calibrated to within +/-0.25°C. As a secondary temperature monitoring system for validation of the temperature reached within the sleeve, test strips (Thermax®, Burlington, NJ) were placed in the mesh sleeve in the EDS test location. The test strips have a surface temperature indicator range of 65°-93°C or 149°-199°F.

**Validation for Vial and Outer Pouch Integrity.** Vials filled with PBS were vacuum-sealed in the outer pouch, then inserted into weighted sleeves and attached to the cap of the sample port, then suspended into the EDS tank. The vials were exposed to the complete EDS cycle. The EDS cycle consisted of fill, warm up, heat treatment (82.2°C for 6 hours at 5 psi), and cooling. Next, the vials and the sealed outer plastic pouch barrier were examined for volume change and evidence of leak or evidence of loss of structure integrity.

**Preparation of Virus Samples and Re-isolation Procedures After EDS Treatment**

**Avian Influenza Virus and Avian Paramyxovirus.** The enveloped viruses, LPNAI/NY/94, HPAI/PA/83, I/NDV-B1/48, and vNDV/CA/2002 (Table 1), were propagated by inoculation of 10-day-old specific pathogen free (SPF) embryonating white leghorn chicken eggs (ECE) (Southeast Poultry Research Laboratory, Athens, GA). Titration of the virus stock was completed using standard methods (Swayne et al., 1998). Briefly, 10-fold serial dilutions of infected allantoic fluid were inoculated into Brain Heart infusion broth (BHI, Becton Dickinson, Sparks, MD) containing antibiotics (gentamicin 200 μg/ml, penicillin 2000 units/ml, amphotericin B 4 mg/ml, Sigma Chemical Co., St. Louis, MO). One hundred microliter aliquots were then inoculated into five SPF eggs. Virus titer was determined by calculation of the 50% egg infectious dose (EID₅₀) (Reed et al., 1938). In preparation for the EDS treatment, an initial titer of 6 log₁₀ EID₅₀ was used for avian influenza viruses (HPAI and LPNAI) and avian paramyxovirus (vNDV and iNDV). After EDS treatment, AV and NDV were re-isolated in accordance with established procedures (Alexander, 2005; Senne, 2008). Briefly, samples were inoculated into five 9-to-10-day-old chicken embryos by the chorioallantoic sac (CAS) route. Following the 4 days of incubation, allantoic fluids from each embryo were harvested and tested for hemagglutination of RBC. Pooled allantoic fluids were inoculated into embryos for a second passage and then retested for the presence of virus using the hemagglutination assay. The positive control samples were titrated using the hemagglutination assay.

**Avian Metapneumovirus.** aMPV subtype C Colorado strain (aMPV-CO) (Table 1) was propagated in Vero cells as described previously (Yu et al., 2010). An initial titer of 7.5 log₁₀ 50% tissue culture infectious dose (TCID₅₀) of aMPV-CO was used for EDS treatment. The re-isolation of avian metapneumovirus following EDS treatment was in accordance with established procedures for aMPV detection (Yu et al., 2010). Briefly, each of the three replicates of the virus and control vial was serially diluted in D-MEM cell culture medium and plated in triplicate onto confluent Vero cells in a 96-well format. Cell culture plates were monitored each day for 5 days for typical cytopathic effects (CPE). Viral titers of the EDS treated and untreated aMPV-CO samples were calculated and compared by the TCID₅₀ (Reed et al., 1938).

**Marek’s Disease Virus Type-1.** MDV-1/CV1988 (Table 1) was propagated in chicken embryo fibroblast (CEF) cells supplemented with media (DMEM/0.2% FBS) and incubated at 39°C with 5% CO₂ in a humidified atmosphere for 4-6 days. Following infection (based on visual CPE), infected cells were trypsinized, pelleted for 5 minutes at 300 x g, and resuspended in freezng medium (72.5% DMEM; 20% FBS (Gibco®, Invitrogen Corp., Carlsbad, CA), 7.5% DMSO). The cellular suspension was divided into 4 x 1 ml aliquots and slowly cooled to -80°C before transfer to liquid nitrogen for storage. Following EDS treatment, treated samples, along with the untreated control, were serially diluted in DMEM containing 5% FBS (10⁻²-10⁻³). Each dilution was used to infect one well (24-well) of CEF at ~90% confluency in DMEM with 5% FBS. After approximately 12 hours, the medium was removed and replaced with DMEM/0.2% FBS, and the infected cells were incubated at 39°C with 5% CO₂ humidified atmosphere for an additional 4 days. Cells were then fixed with a 50:50 mixture of acetone/methanol, and viral foci were enumerated using immunohistochemistry with a monoclonal antibody specific for glycoprotein B of MDV-1. Viral titers of treated and untreated samples were calculated and compared.

**Avian Parvovirus.** Aliquots of 5 x 1 ml ChPV-P1 (Table 1) stock virus (6 log₁₀ infectious particle per ml) were added to 5 x 4 ml internally threaded cryogenic vials (Corning #430491, Lowell, MA). Sterile PBS was added to four cryovials to serve as negative controls. Three replicates were immersed in the EDS system, while two replicates of each virus were maintained at -80°C during the process to serve as a positive control. Following EDS inactivation, the test vials were stored at -80°C prior to testing. Since chicken parvovirus (ChPV) does not grow in vitro, the safety test was evaluated
in vivo in chickens. Four hundred microliters of treated virus from each of three vials were pooled for testing. Ten 1-day-old SPF chickens were orally inoculated with 100 µl of pooled treated virus. Positive control birds (n=10) received 100 µl of untreated virus. Cloacal swab samples were taken at 7, 14, and 21 days post inoculation from each test sample and control birds. DNA was extracted from cloacal swab samples (DNA Extraction Kit, Qiagen, Germantown, MD) and processed using a chicken parvovirus-specific PCR assay (Zsak et al., 2009).

Avian Reovirus and Rotavirus. Reo/TX/98 and Ro/996/07 viruses (Table 1) were propagated and prepared for EDS treatment as previously described for avian influenza (Spackman et al., 2005; Theil et al., 1986). Following EDS treatment, each of the three replicates per virus and control vial were serially diluted in culture medium (DMEM with antibiotics) and plated in triplicate onto VERO cells (reovirus) or MA-104 cells (rotavirus) grown to approximately 80% confluency in 96-well plates. Cell culture plates were monitored daily for 5 days for CPE typical of reovirus and rotavirus.

Astrovirus. TAsTV/07 (Table 1) was propagated and prepared for EDS treatment as previously described (Schultz-Cherry et al., 2001; Spackman et al., 2005). Following EDS treatment, three replicates were inoculated into ten 20-day-old SPF embryonating turkey eggs via the yolk sack. Eggs were candied daily for 7 days to check for mortality. When the eggs reached 27 days of incubation, intestines were harvested, diluted (1:2), and homogenized. RNA was extracted from the homogenate using a Trizol-MagMax procedure and tested via RT-PCR for the presence of turkey astrovirus RNA (Das et al., 2009; Day et al., 2007). Control virus held at -70°C served as a positive control and was tested in an identical manner.

Salmonella Enterica Serovar Enteritidis. Salmonella enterica serovar Enteritidis was propagated into tryptone soya broth and incubated for 24 hours at 37°C, centrifuged for 10 minutes at 3,000 x g, then washed twice in 0.85% saline. The cell concentration was determined spectrophotometrically at O.D. 600 nm and adjusted to a final cell concentration of 7 log10 cfu/ml. Two milliliters of culture were added to eight vials. Four vials were immersed into the EDS system, while the remaining vials were maintained at -70°C and served as positive controls. Following EDS treatment, the cell count (cfu/ml) from treated and control vials was determined by serial dilution in saline, then enumeration on brilliant green and trypticase soy agar. Negative control vials containing PBS were processed as above.

Results and Discussion

Enveloped viruses (AI virus, AMV-1, aMPV, and Marek's disease virus type-1) and non-enveloped viruses (ChPV, TAsTV, reo- and rotaviruses) and Salmonella have had detrimental economic impact in the poultry industry worldwide; therefore, research on avian pathogens is essential to find control measures. These pathogens are handled in the animal health research facility environment including isolation cabinets, open-housing rooms, and necropsy facilities. The pathogens accumulate during room clean up with some quantities amassing in effluent tanks. HPAIV and vNDV are Select Agents and as such are handled in a high-containment facility (BSL-3E) and require effluent inactivation. The others are BSL-2 pathogens and are not required to be inactivated in effluent; however, our facility chose to also inactivate these in the effluent prior to release to the municipal waste. Enveloped and non-enveloped viruses have different thermal resistances. Non-enveloped viruses are usually more heat-resistant than enveloped viruses (Blümel et al., 2002). The non-enveloped parvovirus has a single-stranded (ss) DNA and is one of the most thermostable viruses. Studies by Emmoth and others showed that swine parvovirus heated at 70°C for 60 minutes resulted in 2.6 to 3.2 log10 titer reduction, while Alexander et al. (2004) demonstrated that enveloped ss RNA Newcastle disease virus could be inactivated (1 log10) at 70°C within 82 seconds (Blümel et al., 2002; Emmoth, 2010). Heating medium influences heat sensitivity for some viruses (Yunoki et al., 2003). For example, enteroviruses in fresh sludge can be inactivated (5 log10 reduction) following heat exposure of 60°C for 30 minutes, while in digest sludge 4 log10 reduction occurred within 4 minutes (Foliguet et al., 1972; Mocé-Lilvina et al., 2003).

Thermal Inactivation Modeling

Thermal Inactivation Models for Representative Enveloped and Non-enveloped Viruses. Thermal inactivation curves were developed using AI viruses and NDV as representatives of enveloped RNA viruses. These viruses included the highly virulent forms which are Select Agents that are handled and used under BSL-3E and ABSL-3E conditions. Also, low-virulent forms of these viruses (LPNAI including H5 and H7, and NDV) which are BSL-2 viruses were included in these studies. Thermal inactivation curves were generated for HPAI/PA/83 (H5N2) and resulted in a thermal death rate as presented in Table 2. Heat exposure at 59°C resulted in 5 log10 reduction within 6 minutes while exposure to heat treatment at 70°C-82.2°C resulted in total inactivation (6.6 log) in less than 30 seconds. The thermal inactivation data (D, and zd values) for LPNAI/NY/94 (H7N2), presented in Table 3, show that as the temperature increased, LPNAI virus survival declined exponentially, inactivating 5 log10 at 59°C within 5.5 minutes of heat exposure and total inactivation (6.25 log10) at 70°C-82.2°C in less than 30 seconds.

Thermal inactivation for vNDV/CA (Table 4) at 59°C required at least 10 minutes and at 70°C at least 5.5 minutes for a 6 log10 viral reduction, while heat exposure at 82.2°C resulted in 8.8 log10 reduction in less than 30 seconds. For NDV/B1 (Table 5) exposure at 59°C
Table 2

$D_t$ values (time required to reduce virus titer by 90% or 10$^1$ TCID$_{50}$) with standard deviation generated from predictive thermal inactivation equation of HPAI-A/ck/PA/1370/83 (H7N2).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$D_t$ Value (min)</th>
<th>SD</th>
<th>Equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>12</td>
<td>+/-0.005</td>
<td>$y = -0.0837x + 6.3116$</td>
<td>0.96</td>
</tr>
<tr>
<td>57</td>
<td>4.8</td>
<td>+/-0.01</td>
<td>$y = -0.2105x + 5.7903$</td>
<td>0.96</td>
</tr>
<tr>
<td>58</td>
<td>2.3</td>
<td>+/-0.031</td>
<td>$y = 5.9696e^{-0.1034x}$</td>
<td>0.99</td>
</tr>
<tr>
<td>59</td>
<td>1.3</td>
<td>+/-0.21</td>
<td>$y = 0.0444x^2 - 0.861x + 5.0173$</td>
<td>0.97</td>
</tr>
<tr>
<td>61</td>
<td>1.0</td>
<td>+/-0.09</td>
<td>$y = -0.969x + 3.436$</td>
<td>0.85</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$zD$ Value</td>
<td>5.3°C</td>
<td>+/-0.03</td>
<td>$y = -0.1883x + 11.372$</td>
<td>0.93</td>
</tr>
</tbody>
</table>

1 Infected with an initial titer of 6.4 log (TCID$_{50}$/ml) in PBS.
2 Experiment was replicated three times.
3 The correlation coefficient ($R^2$) indicated the goodness of fit of the predictive equation.
4 ND—6.4 log$_{10}$ reduction upon reaching 70°C at time zero.
5 ND—6.4 log$_{10}$ reduction upon reaching 82.2°C at time zero.

Table 3

$D_t$ values (time required to reduce virus titer by 90% or 10$^1$ TCID$_{50}$) with standard deviation generated from predictive thermal inactivation equation of LPNAI A/ck/NY/13142-5/94.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$D_t$ Value (min)</th>
<th>SD</th>
<th>Equation</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>21.7</td>
<td>+/-0.58</td>
<td>$y = -0.0461x + 5.4767$</td>
<td>0.61</td>
</tr>
<tr>
<td>55</td>
<td>2.8</td>
<td>+/-0.32</td>
<td>$y = -0.3473x + 4.0462$</td>
<td>0.87</td>
</tr>
<tr>
<td>57</td>
<td>2.2</td>
<td>+/-0.78</td>
<td>$y = -0.4457x + 3.22$</td>
<td>0.84</td>
</tr>
<tr>
<td>58</td>
<td>1.2</td>
<td>+/-0.28</td>
<td>$y = -0.8817x + 4.74$</td>
<td>0.96</td>
</tr>
<tr>
<td>59</td>
<td>1.1</td>
<td>+/-0.30</td>
<td>$y = -0.7913x + 3.2837$</td>
<td>0.99</td>
</tr>
<tr>
<td>61</td>
<td>0.8</td>
<td>+/-0.58</td>
<td>$y = -1.1167x + 2.616$</td>
<td>1.0</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>82.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$zD$ Value</td>
<td>5.7°C</td>
<td>+/-0.04</td>
<td>$y = -0.1575x + 9.353$</td>
<td>0.80</td>
</tr>
</tbody>
</table>

1 Infected with an initial titer of 6.25 log$_{10}$ (TCID$_{50}$/ml) in PBS.
2 Experiment was replicated three times.
3 The correlation coefficient ($R^2$) indicated the goodness of fit of the predictive equation.
4 ND—6.25 log$_{10}$ reduction upon reaching 70°C at time zero.
5 ND—6.25 log$_{10}$ reduction upon reaching 82.2°C at time zero.

resulted in the inactivation of 6 log$_{10}$ viral titer within 16 minutes of heating; at 70°C there was a 6 log$_{10}$ reduction within 5 minutes, and at 82.2°C, a greater than 6 log$_{10}$ reduction of NDV/B1 occurred within less than 30 seconds. Previous research supports our observation that AI viruses can be inactivated rapidly at temperatures above 59°C. Thomas and colleagues (2008) observed the rapid inactivation of AI and NDV (7 log$_{10}$ reduction) in chicken meat within 5 seconds of cooking at 73.9°C. The change in thermal resistance data ($zD$) demonstrated that HPAI/PA/83 and LPNAI/NY/83 (Tables 2 and 3) had the most thermal resistance followed by vNDV/CA (Table 3), then NDV/B1 (Table 4). The $zD$ values for HPAI/PA/83 and LPNAI/NY/94 suggest that although they differ in being of high- and low-pathogenicity types, respectively, they had similar change in thermal resistance. vNDV was more thermally resistant than NDV up to 70°C, but at 82.2°C NDV demonstrated slightly more thermal resistance. Ro/1145/08 and Reo/TX/99 were used to develop
Table 4

\( D_t \) values (time required to reduce virus titer by 90% or 10\(^1\) TCID\(_{50}\)) with standard deviation generated from predictive thermal inactivation equation of vNDV Chicken/CA/212676/2002.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>( D_t ) Value (min)</th>
<th>SD</th>
<th>Equation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>11.2</td>
<td>+/-0.6</td>
<td>( y = -0.0893x + 8.7735 )</td>
<td>0.97</td>
</tr>
<tr>
<td>56</td>
<td>6</td>
<td>+/-0.4</td>
<td>( y = -0.1694x + 8.7401 )</td>
<td>0.99</td>
</tr>
<tr>
<td>57</td>
<td>3.1</td>
<td>+/-0.37</td>
<td>( y = 8.6295e^{-0.0435x} )</td>
<td>0.98</td>
</tr>
<tr>
<td>58</td>
<td>3.1</td>
<td>+/-0.03</td>
<td>( y = 0.0149x^2 - 0.6161x + 8.8421 )</td>
<td>0.992</td>
</tr>
<tr>
<td>59</td>
<td>1.5</td>
<td>+/-0.09</td>
<td>( y = 0.0193x^2 - 0.7107x + 8.00 )</td>
<td>0.93</td>
</tr>
<tr>
<td>70</td>
<td>0.91</td>
<td>+/-0.08</td>
<td>( y = -0.74x + 1.5 )</td>
<td>1</td>
</tr>
<tr>
<td>82.2</td>
<td>ND(^4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>z( D ) Value</td>
<td>4.9</td>
<td>+/-0.02</td>
<td>( y = -0.2033x + 12.186 )</td>
<td>0.95</td>
</tr>
</tbody>
</table>

1 Infected with an initial titer of 8.8 log\(_{10}\) (TCID\(_{50}\)/ml) in PBS.
2 Experiment was replicated three times.
3 The correlation coefficient (\( R^2 \)) indicated the goodness of fit of the predictive equation.
4 ND—8.8 log\(_{10}\) reduction within 30 seconds at 82.2°C.

Table 5

\( D_t \) values (time required to reduce virus titer by 90% or 10\(^1\) TCID\(_{50}\)) with standard deviation generated from predictive thermal inactivation equation of iNDV Chicken/U.S./B1/1948.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>( D_t ) Value (min)</th>
<th>SD</th>
<th>Equation</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>4.4</td>
<td>+/-0.02</td>
<td>( y = 9.0336e^{-0.0352x} )</td>
<td>0.82</td>
</tr>
<tr>
<td>55</td>
<td>3.2</td>
<td>+/-0.03</td>
<td>( y = 7.5789e^{-0.0394x} )</td>
<td>0.89</td>
</tr>
<tr>
<td>57</td>
<td>2.8</td>
<td>+/-0.05</td>
<td>( y = 8.0925e^{-0.1513x} )</td>
<td>0.97</td>
</tr>
<tr>
<td>58</td>
<td>2.9</td>
<td>+/-0.10</td>
<td>( y = 6.0713e^{-0.2132x} )</td>
<td>0.88</td>
</tr>
<tr>
<td>59</td>
<td>2.7</td>
<td>+/-0.12</td>
<td>( y = 4.7738e^{-0.1146x} )</td>
<td>0.8</td>
</tr>
<tr>
<td>61</td>
<td>1.95</td>
<td>+/-0.1</td>
<td>( y = 6.5755e^{-0.2732x} )</td>
<td>0.96</td>
</tr>
<tr>
<td>70</td>
<td>0.82</td>
<td>+/-0.15</td>
<td>( y = 2.84x^2 - 4.46x + 2.95 )</td>
<td>1</td>
</tr>
<tr>
<td>82.2</td>
<td>ND(^4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>z( D ) Value</td>
<td>0.92</td>
<td>+/-0.05</td>
<td>( y = 264.35e-0.0792 )</td>
<td>0.97</td>
</tr>
</tbody>
</table>

1 Infected with an initial titer of 9.8 log\(_{10}\) (TCID\(_{50}\)/ml) in PBS.
2 Experiment was replicated three times.
3 The correlation coefficient (\( R^2 \)) indicated the goodness of fit of the predictive equation.
4 ND—9.8 log\(_{10}\) after 30 seconds of heat exposure at 82.2°C.

Anisothermal models to represent non-enveloped, double-stranded RNA viruses in the PBS model system. The survival data for Ro/1145/08 and Reo/TX/99 following anisothermal heat treatment at 25°C to 82.2°C for 140 seconds indicated that these viruses were more heat-resistant than AI viruses and NDV, requiring exposure of more than 2 minutes at 82.2°C to inactivate 5 log\(_{10}\) TCID\(_{50}\)/ml of both viral populations. No virus was recovered for Ro/1145/08, Reo/TX/99 (5.3-5.8 log\(_{10}\) TCID\(_{50}\)/ml), and TAstV/07 following heat exposure at 82.2°C for 140 seconds (2.3 minutes) (Table 6). Previous studies of survival and infectivity of reovirus and rotavirus following heat exposure determined that rotavirus was rapidly inactivated at 50°C in 30 minutes with only 1% survival while 50% of a 4x10\(^7\) pfu/ml population of reovirus survived similar heat exposure (Estes et al., 1979).

The heat inactivation method of Reo/TX/99 and Ro/1145/08 used anisothermal heating gradient; therefore, thermal death rate at specific temperatures (\( D_t \)) or the change in thermal resistance (z\( D \)) could not be calculated. Nevertheless, thermal resistance or stability were observed in the anisothermal curve between 25°C-50°C.

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Table 6
Survival of Reovirus TX/99, Rotavirus 1145, and astrovirus following heat exposure from 25°C to 82.2°C over a ramping up time of 0 to 140 seconds with no holding time using a thermocycler. Astrovirus heated at 82.2°C for 30 minutes.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (sec)</th>
<th>Titer (Log EID50/ml) Reovirus TX/99</th>
<th>Titer (Log EID50/ml) Rotavirus 1145</th>
<th>Survival of Astrovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>10</td>
<td>5.33</td>
<td>5.8</td>
<td>9/10</td>
</tr>
<tr>
<td>40</td>
<td>35</td>
<td>4.73</td>
<td>5.73</td>
<td>—</td>
</tr>
<tr>
<td>50</td>
<td>60</td>
<td>4.73</td>
<td>5.77</td>
<td>—</td>
</tr>
<tr>
<td>60</td>
<td>85</td>
<td>4.03</td>
<td>3.67</td>
<td>7/10</td>
</tr>
<tr>
<td>70</td>
<td>110</td>
<td>&lt;1.6</td>
<td>2.1</td>
<td>0/9</td>
</tr>
<tr>
<td>82.2</td>
<td>140</td>
<td>&lt;1.6</td>
<td>&lt;1.8</td>
<td>0/10</td>
</tr>
<tr>
<td>82.2</td>
<td>30 minutes holding time</td>
<td>—</td>
<td>—</td>
<td>0/10</td>
</tr>
<tr>
<td>zD (°C)</td>
<td>—</td>
<td>1.5</td>
<td>1.2</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Astrovirus was detected in turkey embryo intestinal homogenates via RT-PCR.

followed by an exponential rate of decline between 50°C and 82.2°C. This trend could be supported by previous research which showed an initial lag phase between 45°C-50°C followed by a rapid exponential decline as temperature increased (Middleton et al., 2002). The thermal inactivation data suggest that Ro/1145/08 is more thermostable than Reo/TX/99 (Table 6). The thermal death rate (D) for both AlV and NDV viruses was between 1.1-2.7 minutes at 60°C, and less than 30 seconds at 82.2°C. The thermal inactivation for Reo/TX/99 and Ro/1145/08 was greater than a 4 log10 reduction within 2 minutes upon reaching 82.2°C. Therefore, to obtain the 6 log10 sterility assurance level required, less than 30 seconds of heating at 82.2°C for Al (HPAI/PA/83, LPNAI/NY/94, and vNDV/CA/2002, /NDV/B1/48), 1.8 minutes for TAstV/07, and at least 3 minutes for Reo/TX/99 and Ro/1145/08 viruses are required.

EDS Validation of Enveloped and Non-enveloped Viruses

Thermal inactivation is an effective means of inactivating pathogens including viruses in liquid or sludge. In many facilities, the effluent is collected in tanks and exposed to indirect steam heating prior to discharge into the public sewerage. The Clean Water Act (CWA) of 1972 regulates water quality and the standard for the disposal of waste sludge set the standard for pathogen level in sludge waste, and for enteric viruses only 1PFU/4g sludge is acceptable (Anonymous, 1972; Anonymous, 1993). The Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual recommends that thermal inactivation processes for laboratory waste (such as EDS) should ensure a sterility assurance level (SAL) of a 6 log reduction (CDC, 2007). In our validation study, enveloped and non-enveloped viruses were exposed to indirect steam heating at 82.2°C for 6 hours using static EDS. For our final validation tests, no viable virus was recovered from any of the submerged vials of viruses in the EDS tanks (Table 7).

Enveloped Viruses

Avian metapneumovirus (aMPV) is relatively heat-labile and is routinely inactivated (> 3 log10) in laboratory procedures following 30 minutes of heat exposure at 56°C or 60°C for 10 minutes or autoclaving at 121°C (15 psi) for 20 minutes (Calnek et al., 1971; Cook, 2000; Elhai, et al., 2004). Marek’s disease virus is an avian cell-associated virus and is extremely sensitive to environmental conditions so most research information is limited (Jarosinski et al., 2007; Nazerian et al., 1970). However, research on a related alphaherpes virus, pseudorabies virus (PrV) which is cell-free, shows that it can be inactivated by 7.1 log10 pfu/ml following pasteurization in milk (60°C for 30 minutes), and by 6.6 log10 pfu/ml in plasma following steam treatment (60°C for 10 hours plus 80°C for 1 hour) (Chang et al., 2010; Sofer, 2003). Our EDS process completely inactivated 7.5 log10 aMPV TCID50/ml and 9 log10 pfu/ml MDV, so inactivation was greater than the SAL requirement for EDS by at least 1.3-fold.

Avian influenza viruses (LPNAI/NY/94 and HPAI/PA/83) and Newcastle disease viruses (NDV/1948/B1 and vNDV/CA/2002) were totally inactivated by the EDS process by greater than the required 6 log10 SAL or more than 1.3 times the SAL requirement (Table 7). The susceptibility of Al viruses and NDV to heat treatment has been demonstrated previously by, showing 7 log10 viral reduction of HPAI Korea/03, vNDV/CA and INDV/Ulster in chicken meat following cooking at 70°C for 5 seconds.
Table 7
The survival of enveloped and non-enveloped RNA and DNA viruses following the exposure to the effluent decontamination system (EDS) for 6 hours at 82.2°C.

<table>
<thead>
<tr>
<th>Virus Name</th>
<th>Initial Titer (log EID₅₀/ml, TCID₅₀/ml or infectious particle/ml)</th>
<th>Final Titer (log EID₅₀/ml, TCID₅₀/ml or infectious particle/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enveloped Viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avian metapneumovirus Colorado strain</td>
<td>7.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Marek’s disease virus strain Gallid herpesvirus 2 vaccine strain CVI988 (Merial)</td>
<td>9.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Lentogenic avian paramyxovirus strain Chicken/U.S./B1/1948 (B1)</td>
<td>8.2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Velogenic avian paramyxovirus strain CK/U.S.(CA)/212676/2002 (S0212676)</td>
<td>8.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Highly pathogenic avian influenza virus strain A/chicken/PA/1370/1983 (H5N2)</td>
<td>6.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Low pathogenic notifiable avian influenza virus strain A/chicken/NY/13142-5/1994 (H7N2)</td>
<td>7.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Non-enveloped Viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reovirus strain Turkey/TX/SEP/98</td>
<td>11.1</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>Rotavirus strain Turkey/NC/SEP-Ro996/07</td>
<td>5.1</td>
<td>&lt;1.1</td>
</tr>
<tr>
<td>Astrovirus strain Turkey/CA/SEP-A938/07</td>
<td>8/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Avian parvovirus strain chicken parovirus, ChPV-P1</td>
<td>6.0</td>
<td>&lt;1</td>
</tr>
<tr>
<td><strong>Vegetative Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella enterica Enteritidis</td>
<td>CFU/ml</td>
<td>CFU/ml</td>
</tr>
<tr>
<td></td>
<td>9.1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Other investigations showed similar log reduction in liquid egg products in less than 2.5 minutes at 63°C (Swayne et al., 2004; Thomas et al., 2007; Thomas et al., 2008).

**Non-enveloped Viruses**
Non-enveloped viruses are usually more thermally resistant than enveloped viruses at the temperature range of 54°C-61°C (Blümel et al., 2002). Reovirus is more thermostable than rotavirus and both can survive and remain infectious following heat treatment at 50°C for 30 minutes (Estes et al., 1979). Recent work (Middleton et al., 2002) demonstrated that heating at 62°C for 30 minutes was required to inactivate 5 log pfu/ml. Astrovirus is highly thermostable and is difficult to titer at low concentration; therefore, for the EDS safety test the enumeration process was based on a fraction negative calculation. The initial inoculum of TAstV/07 was positive in 8/10 of the inoculated eggs. Following the EDS heat processing, all inoculated eggs were negative for astrovirus (Table 7). Astrovirus associated with Poul Enteritis Mortality Syndrome is considered relatively heat-resistant, surviving heat exposure at 60°C for 10 minutes (Richards, 2001; Schultz-Cherry et al., 2001).

In the EDS validation process (82.2°C for 6 hours), 6 log₁₀ infectious dose/ml of chicken parvovirus (ChPV-P1) was totally inactivated as determined by in vivo tests (Table 7). Parovirus is very thermostable and animal paroviruses are more heat-stable than human parovirus. Animal paroviruses can resist heat exposure of 60°C for 30 minutes, and some strains of bovine parovirus (BPV) can be heat-resistant up to 100°C dry heat for 30 minutes (Bräuniger et al., 2000; Yunoki et al., 2003). As an example, exposure of bovine parovirus (BPV) to dry heat between 40°C and 95°C for 1 hour resulted in inactivation of less than 0.8 log₁₀ TCID₅₀/ml, and for 2-hour incubation only 0.9 log₁₀ TCID₅₀/ml was inactivated (Sauerbrei et al., 2009). In some strains of BPV, infectivity can be maintained for 4 days at 56°C or for 8 hours at 70°C (Durham et al., 1985). Animal parovirus can survive the pasteurization process for human serum albumin at 60°C for 10 hours (Blümel et al., 2002). One of the features of the heat inactivation of parovirus is that the DNA is released from an intact capsid, thereby losing its ability to infect a host (Sauerbrei et al., 2009).

**Salmonella**
Following the EDS validation process (82.2°C for 6 hours), 9.1 log₁₀/ml of Salmonella enterica serovar Enteritidis was totally inactivated (Table 7). The EDS process should adequately inactivate Salmonella as shown in previous research where thermal death rate (D₅₀) for Salmonella spp. in ground chicken meat ranged from...
43.3 minutes at $D_{55}$ to 0.07 minutes at $D_{76}$ with a change in thermal resistance ($zD$ values) of 5.3°C, and in homogenized whole eggs with thermal inactivation rates of 5.7 minutes at $D_{54}$, and 0.17 minutes at $D_{60}$ with $zD$ value of 3.95°C (Jin et al., 2008; Murphy et al., 2004). The log reduction for Salmonella in the EDS process was more than 1.5-fold higher than the SAL requirement.

This validation of a moderate temperature (82.2°C for 6 hours) EDS process verifies the effectiveness of the inactivation of both enveloped and non-enveloped RNA and DNA viruses, and Salmonella in liquid media showing greater than the 6 log$_{10}$ reduction required by the Clean Water Act for wastewater discharge (Anonymous, 1972). However, fecal pathogens have been shown to survive better in solid waste than in liquid waste, suggesting that waste with a high content of organic matter may require longer thermal treatments times for inactivation of pathogens (Mocé-Lilivina et al., 2003; Yunoki et al., 2003). In our effluent decontamination system, the effluent had low quantities of organic matter, supporting the validity of our model system and the lack of a need to develop models that included high organic matter content. Our validation process indicates the following to support effective effluent decontamination from an avian pathogen research facility: 1) greater than 6-log$_{10}$ EID$_{50}$ or TCID$_{50}$/ml reduction for enveloped single-stranded RNA viruses (LPNAI/NY/94, HPAI/PA/83, iNDV/B1, vNDV/CA/2002, and aMPV) and enveloped double-stranded DNA virus (Marek's disease virus CV988); 2) greater than 9 log$_{10}$ cfu/ml reduction of a vegetative bacterial pathogen (Salmonella enterica serovar Enteritidis); 3) greater than 5 log$_{10}$ and 11 log$_{10}$ TCID$_{50}$/ml reduction for non-enveloped double-stranded RNA viruses (rotavirus Ro/996/07) and reovirus Reo/ TX/98, respectively; 4) no recovery of non-enveloped positive sense RNA virus (astrovirus TASTv/07) using 10 embryonating turkey eggs; and 5) at least 6 log$_{10}$ reduction in non-enveloped single-stranded DNA virus (chicken parvovirus ChPV-P1). This assures that the effluent released to the municipal wastewater system is free of bacterial and viable viral pathogens (Table 7).

**Conclusion**

The heat inactivation models predicted that the reduction of 6 log$_{10}$ TCID$_{50}$/ml of the BSL-3 agents (HPAI/PA/83 (H5N2) and vNDV/CA/2002) would occur within 30 seconds at 82.2°C; therefore, the EDS treatment at 82.2°C for 6 hours has a margin of safety 720-fold higher than is required. The BSL-2 agents would require at least 3 minutes to inactivate 6 log$_{10}$ virus TCID$_{50}$/ml, so the EDS treatment would be 120-fold higher than is necessary.

The EDS tank inactivation system demonstrated that the enveloped and non-enveloped RNA and DNA viruses and Salmonella would be inactivated within the 6 hours at 82.2°C, giving a significant margin of safety. The EDS validation process confirms that the 6 log reduction of pathogens required for sanitary assurance level for effluent release to the municipal waste system can be achieved.

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Laboratory Safety for Oncogene-Containing Retroviral Vectors

John T. Gray*
St. Jude Children’s Research Hospital, Memphis, Tennessee

Abstract

The use of retroviral vectors in research laboratories has been steadily increasing, and with that, questions are arising more frequently regarding the biosafety of these reagents, particularly when the vector delivers a gene with oncogenic properties. Although modern retroviral vector systems produce particles that are replication-defective, there is still a possibility that upon accidental exposure, cells within a laboratory worker could be permanently modified and that this may increase the risk of malignant transformation. This brief review summarizes some published research that bears relevance to this risk and is intended to help investigators, IBC staff, and occupational health professionals establish policies to minimize the risk of working with these reagents. Questions regarding the actions that should be taken to prevent and respond to accidental exposure are presented and discussed.

Introduction

Retroviral vectors can permanently alter cellular genomic DNA by inserting a gene expression cassette into the chromosome and, as such, are valuable tools for studying the effect of genes on the growth and differentiation of cells. This tool is often implemented in cancer research by modifying cells ex vivo to express candidate oncogenes, followed by reintroduction of the modified cells into animals and scoring subsequent tumor development. In this context, the risk of accidental exposure with an oncogenic vector to laboratory personnel should be considered with regard to the potential that the vector could transduce tissue in vivo and facilitate a transformation event, leading to an increased risk of cancer over the life of the exposed individual. This risk occurs in spite of the fact that the most common retroviral vectors are replication defective, and they cannot undergo more than a single round of transduction. They are, therefore, not truly infectious agents, as they cannot establish a productive infection in an individual that is capable of spreading into the general population. The risk of tumorigenesis with the use of oncogene-containing vectors should, therefore, be evaluated as potentially carcinogenic substances rather than hazardous infectious agents. It is possible for some retroviral vector production systems to sporadically generate replication-competent retrovirus contaminants, which could potentially create a spreading, tumorigenic virus infection, but modern vector production systems have reduced these contaminants to undetectable levels. As most investigators use these safer production systems, this discussion is specifically limited to the risk associated with fully replication-defective vectors. For a review of general retrovirus vector safety, not focused on oncogene-containing vectors, see Mosier (2004).

This review addresses the safety of oncogene-containing vectors by first explaining the relevant basic retrovirus biology, and then trying to provide examples of published data that will help evaluate the likelihood that an accidental exposure to a laboratory worker would: 1) cause significant levels of transduction; and 2) at some level of transduction would increase the risk of cancer. These risks are then discussed in the context of developing institutional policies for managing work with these types of reagents.

Retrovirus Vector Basics

To evaluate the potential risk of accidental transformative events, it is necessary to understand some basic concepts of retroviral vectorology. For further reading on retroviruses and their use as vectors, please refer to Retrovirology (Coffin, 1997), available free online via the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Retroviral vectors are produced in immortalized cell lines engineered to express: 1) the necessary protein-coding genes to generate viral particles; and 2) a vector RNA genome that is packaged into the viral particle. These “producer cells” secrete vector particles into the medium and are typically processed by filtration or optionally by column purification or ultracentrifugation techniques. Retroviral vector concentration is typically determined by an infectious titration assay on a standard immortalized cell line, such as Hela or HEK-293, by determining the number or fraction of cells that have the vector genome integrated (a “transduction” event) after treatment with a given volume of a vector preparation. Typical titers for unprocessed supernatants are between 10^6-10^8 transducing units (TU)/ml, and those of concentrated preparations can extend up to 10^10 TU/ml. The level of gene delivery achieved by a vector preparation (both with regard to the number of cells transduced and the number of transductions per cell) is critically dependent upon the concentration of vector during the incubation of the vector with cells.

Retroviral vectors can be produced with any one of a number of different envelopes, (referred to as vector “pseudotypes”), which each confer distinct cellular tro-
pism. “Ecotropic” envelope vectors can transduce only mouse (and not human) cells, providing enhanced safety for laboratory personnel. The most commonly used pseudotype is the Vesicular Stomatitis Virus glycoprotein G (VSV-G), because it provides high human cell transduction, broad tissue tropism, and excellent particle stability during post-production processing steps, such as ultracentrifugation. After binding to the surface of a cell, retroviral vector particles are internalized, whereupon the genomic RNA is reverse-transcribed to generate a double-stranded DNA molecule competent for integration. The “pre-integration complex” containing this DNA must then gain access to cellular genomic DNA, which in dividing cells can occur as the nuclear envelope breaks down during cell division. Lentiviral vectors, such as those derived from Human Immunodeficiency Virus (HIV), are capable of transducing non-dividing cells by utilizing an active transport process to migrate through the nuclear pore to access the nucleus, providing a distinct advantage of this vector system over traditional gammaretroviral vectors based on Murine Leukemia Virus (MLV). The final step of the transduction process occurs when the viral integrase enzyme mediates integration of the proviral DNA into the genome. The efficiency of the entire infection process can vary dramatically among different cells. In general, immortalized cells grown in vitro are more easily transduced than primary cells, and resting primary cells are more difficult to transduce than dividing primary cells. Even for lentiviruses, transducing primary cell types can require over 10-fold higher vector titer than is necessary for transducing HEK293 or HeLa cells.

**The Risk that Accidental Exposure Will Cause Significant Transduction**

The transmission of HIV in people and MLV in mice has been studied and shown to typically occur by direct contact with infected fluids such as saliva, semen, or blood. For HIV, there is no evidence of transmission by casual contact or the bites of mosquitoes (Coffin, 1997). However, the Vesicular Stomatitis Virus and other Rhabdoviruses can be transmitted by aerosol inoculation of airway epithelium (Knipe, 2007), and so it is possible that this transmission property could be conferred to VSV-G pseudotyped lentiviral particles. VSV-G pseudotyped lentiviral vectors have been used to transduce murine airway epithelium for gene therapy applications (Stocker et al., 2009), although in that example transduction was observed only when lungs were pre-treated with a natural surfactant. Efficient transduction of human skin epidermis and dermis by both VSV-G and amphotropic MLV pseudotyped lentiviral particles has been observed (Kunicher et al., 2008), in this case by incubating tissue explants in liquid medium containing 10⁷ TU/mL of vector. Transduction of human skin cells was more efficient than that of murine origin. It is not clear whether intact skin would be transduced with similar efficiencies. With regard to accidental exposure to the eye, direct injection of VSV-G pseudotyped lentiviral vectors into porcine corneal pockets created by laser resection techniques did result in significant transduction of corneal stromal cells (Bemelmans et al., 2009), but again it is not clear whether in the absence of the laser resection significant transduction would occur. In that publication the authors do comment (without providing data) that bathing human corneas without laser resection does cause surface cell transduction without transduction of cells beneath the surface.

In the event of an accidental needlestick with lentiviral vectors, it appears more likely that significant transduction would occur. Pan et al. (2002) performed a careful in vivo biodistribution and toxicity study after intravenous administration of a VSV-G pseudotyped lentiviral vector into mice. In this experiment approximately 10⁷ HEK293 transducing units (equivalent to 10 µl of high-titer concentrated vector preparation) were injected via tail vein into 17 normal BALB/c mice. No pathological findings outside of normal were observed for this GFP encoding vector when these animals were sacrificed at 4 and 40 days. Quantitation of vector biodistribution at 4 days post-infusion, as determined by quantitative PCR, showed significant vector genomes in liver (12-59 vector copies per 100 cell genome equivalents, reported hereafter as percent), bone marrow (5%-37%), and spleen (20%-54%). After 40 days, marking had decreased in liver (0.3%-1%) and spleen (0.045%-0.38%) but remained high in the bone marrow (4.7%-22.7%). Flow cytometry analysis for (green fluorescent protein) GFP fluorescence also indicated significant peripheral blood leukocyte transduction at this 40-day time point (average 20.8%), but lower marking at earlier time points (e.g., 0.73% at day 25), suggesting that progenitor transduction in the marrow led to later marking in differentiated lineages.

One might expect that the blood complement system would significantly reduce the level of gene transfer for vectors gaining access to peripheral circulation, although the magnitude of this effect is difficult to predict. VSV-G pseudotyped retroviral vectors in particular have been shown to be inactivated by human blood complement when incubated at 37°C, but the reported effect is variable and at times only a 3-fold reduction was observed (Croyale et al., 2004; DePol et al., 2000). Murine complement has also been shown to inactivate such vectors (Croyale et al., 2004; Pan et al., 2007), in one study to the same extent as human complement, and yet this inactivation was insufficient to prevent the transduction observed in the aforementioned in vivo biodistribution study. Taking into consideration that typical complement inactivation studies utilize prolonged incubations (>30 minutes) in vitro, and that once bound to cells vector particles are protected from complement inactiva-
tion (Pan et al., 2007), we must realize that the complement system will provide at best a partial barrier which will reduce but not eliminate the likelihood that accidental exposure to the circulatory system would lead to significant transduction.

In summary, there is clearly a potential that retroviral vectors used in research laboratories are capable of transducing significant numbers of human cells after accidental exposure. Important factors which will influence the level of transduction that will occur would be the nature of the envelope used to pseudotype the particles, the type of vector (gamma-retroviral vs. lentiviral), the infectious titer of the material exposed to the subject, the duration of the exposure, the particular tissue exposed, and the level of contact with the circulatory system. For surface contact, it might be expected that VSV-G pseudotyped lentiviral vectors would transduce cell numbers only in the hundreds-to-thousands range, while direct inoculation to the circulation could potentially lead to transduction of many more cells.

**The Risk that Accidental Transduction Will Lead to Transformation**

The transformation of normal cells into a malignant state is a complex process that involves both increased proliferation and the inactivation of the normal cellular controls that protect against proliferative stress. The pathways involved in these events depend upon the original cell type, and early events in the transformative process may influence which other pathways would collaborate to drive a cell to full malignancy. In the context of evaluating risk resulting from accidental transduction, it would be impossible to define one standard of risk which would apply to all cell types transduced and all vectors used in cancer research. Rather, some contrasting examples from the literature can be used to illustrate critical factors that may impact the risk of tumorigenesis after transduction occurs.

**Example 1. Leukemia incidence in human gene therapy experiments.** In two separate gene therapy trials for treating X-linked Severe Combined Immunodeficiency (SCID-X1), a gammaretroviral vector expressing the human common γ-chain gene was used to transduce bone marrow-derived CD34+ cells, prior to reintroduction into the patients (autologous transplantation of gene-modified cells). Five of the 20 patients treated in this trial subsequently developed leukemia, and four of these tumors were shown to contain the therapeutic vector inserted into the chromosome near the LMO2 (Limb domain Only 2) gene, directly implicating the transcriptional activation of that gene in the tumorigenic process. The LMO2 gene is critical for hematopoietic development and has been identified as a fusion partner in several T-cell Acute Lymphoblastic Leukemia (ALL) translocations. Aside from the subsequent improvements in gene therapy vector design that this result prompted, it is also relevant to determining the risk of accidental exposure to a retroviral vector expressing the LMO2 gene directly. Recently published quantitation of retroviral integration site preferences have shown that the gamma-retroviral vectors used in these trials appear to integrate near the LMO2 gene approximately 34-fold more frequently than predicted by its size relative to the whole genome (Cattoglio et al., 2010). This means that in the trial, infusion of 10-50 million transduced CD34+ cells contained approximately 10-50 thousand cells with the vector near the LMO2 gene, which caused leukemia in 4 out of 20 people. It must be considered, however, that this trial involved transduction of CD34+ cells, which are enriched for progenitors and stem cells, and that the cells were introduced into severely immunocompromised infants, both of which could have a profound effect on the frequency of tumor formation. Additionally, other very similar trials to treat a different immunodeficiency (adenosine-deaminase deficiency, or ADA-SCID) have not shown any incidence of tumor formation (in spite of documented vector insertions near LMO2), suggesting that the risk of leukemia additionally depends upon other, more complex aspects of the host environment (Cappelli et al., 2010).

**Example 2. Transduction of murine hematopoietic cells with oncogene-containing vectors.** The influence of the developmental status of the transduced cell on the likelihood of tumorigenesis is illustrated in a set of mouse transplantation experiments utilizing gammaretroviral vectors engineered to express one of three oncogenes (LMO2, TCL1, or ΔTrkA, which is a constitutively active mutant of TrkA) (Newrza et al., 2008). In this study, either purified mature T-cells (2x10^7 cells/mouse) or Sca1+ bone marrow cells (5x10^4 cells/mouse) were transduced with vectors and transplanted into RAG1-deficient mice (an immunocompromised host). Remarkably, only the Sca1+ cells, which are enriched for stem and progenitor cells, were able to form leukemias in mice after transduction with oncogene-containing vectors. When GFP-expressing vectors were transduced into Sca1+ cells, or when mature T-cells were transduced with oncogene vectors, no tumors formed in transplant recipients throughout the follow-up period of 284-518 days (using serial transplantation). The Sca1+ cells transduced with oncogene-expressing vectors generated tumors in 100% of the transplanted mice with a latency dependent upon the gene and ranging from 20-400 days. This result provides strong evidence that the likelihood of tumor formation would critically depend upon the developmental status of the cells transduced with oncogene-containing vectors. Vectors with simple reporter genes, however, are unlikely to significantly lead to tumorigenesis, regardless of the cell type transduced.

**Example 3. Mammary tumors after transduction with potently transforming oncogene.** A final example of tumor formation by oncogene vectors is described by
Siwko et al. (2008). This study utilized a lentiviral vector expressing the potently transforming middle T antigen gene from mouse polyoma virus (PyMT), and the vector was directly injected into mammary ducts of immune-competent mice. The lentiviral vectors were pseudo-typed with an avian sarcoma-leukosis virus (ASLV) envelope, which limits infectivity to either avian cells or cells engineered to express the tva envelope receptor, providing enhanced safety for work with this tumorigenic vector. The authors used a transgenic mouse expressing the tva receptor in epithelial cells (K19-tva on a FVBx129 background) and injected $10^3$ infectious units of the PyMT gene-expressing lentiviral vector. Control injections with comparably prepared reporter vectors allowed a rough approximation of the extent of transduction (<400 cells/gland, with six glands injected/mouse). Remarkably, this procedure produced palpable tumors in 10 of 13 evaluable mice with a mean latency of 3.3 months. Southern blotting of tumor tissue confirmed clonal insertions of the retroviral vector in all of 6 tumors tested. The interpretation of this result with regard to issues of biosafety is that it shows that a very small dose of a potent oncogene-expressing vector can be tumorigenic, even in an immunocompetent host. For the sake of comparison, the chemical mutagen ethyl-NTN-sourea (ENU) also causes mammary tumors in similar mouse strains at a comparable efficiency when injected intraperitoneally at ~1 mg ENU per mouse (Kohlepp et al., 2001). Apparently, oncogene-expressing lentiviral vectors can be as tumorigenic as solutions of ENU used for mouse mutagenesis experiments, if not significantly more so.

Consideration of these examples from the literature then implicates several critical factors which will determine the risk of oncogene-containing retroviral vectors to laboratory workers. Most critical among these is the nature of the gene being delivered by the vector, with known oncogenes much more likely to induce tumors than other genes. Each vector should, therefore, be evaluated based upon what is known about the gene or genes contained in it, and the effective risk could be anywhere from relatively harmless at one extreme and more dangerous than potent chemical mutagens at the other. Additional factors are the concentration of the vector and the ability of the vector to transduce cell types susceptible to transformation. Retroviral vectors are prepared at a wide range of concentrations, ranging from $10^5$-$10^{10}$ TU/ml, and so an accidental exposure of a few microliters by needlestick or splash can lead to transduction of either very few or very many cells. Given the enhanced ability of retrovirus to transduce blood cells, exposures to the circulatory system would be expected to result in significantly more transduction than surface exposure. Although lentiviruses have an increased capacity for transduction of stem cells relative to MLV, direct comparison between the two systems with regard to oncogene-containing vector risk has not been described, and other aspects of MLV vector biology, such as their propensity to integrate near growth-promoting genes (Mitchell et al., 2004), warrant that MLV vectors be treated with similar precaution.

**Establishing Prudent and Practical Guidelines for Work with Oncogene-Containing Retroviral Vectors**

The summary of the above experimental data and concepts is intended to facilitate determination of the optimal best practice with regard to balancing the potential risk of inadvertent exposure to an oncogene-containing vector with the need to use these valuable tools in the research laboratory. Even if such an accidental exposure never occurs, with the increased number of researchers using these reagents it is certainly possible that over multiple decades one or more of these researchers will get cancer (from independent or natural causes), and it may become important for the institution to confirm that the work with the tumorigenic vector was not in any way responsible. Additionally, if reasonable precautions can be taken to reduce the risk of inadvertent exposure or beneficial prophylactic treatment options can be in place should an accidental exposure occur, then it is important to discuss and implement these precautions and procedures proactively.

The potential for potently transforming oncogene vectors to induce tumorigenesis at very low doses requires that personnel working with this select type of vector be educated as to this risk. Along with this precaution, special attention should be paid to work with these vectors when they are used at very high titer and with sharp instruments that could cause blood exposure (such as syringes with needles). Although this highest risk category might apply only to a few genes, such as PyMT and cancer-associated chromosomal truncation and fusion genes, the potential for genes with milder transformative properties to increase a worker’s risk of cancer over the long-term is difficult to quantitate. As such, an open policy of educating all workers involved regarding what is known and not known about these risks is both fair and prudent.

In the event that an accidental exposure occurs, other measures that might ameliorate the potential harmful effects should be considered. Although the retroviral vectors in question perform only a single cycle of transduction into target cells, it is possible that this process could be attenuated by the use of anti-retroviral therapeutics, provided they are administered immediately following exposure. Careful kinetic analysis of HIV transduction kinetics *in vitro* has shown that reverse transcription and integration of the proviral genome is a relatively slow process, requiring between 12 and 24 hours post-infection in primary human CD4+ cells (Vatakis et al., 2009). The speed of transduction is dependent upon the duration of stimulation performed *in vitro*, as reverse transcription occurred more slowly in
less activated cells. Additional experiments showed that addition of the HIV reverse transcriptase inhibitors zidovudine (AZT) and efavirenz (EFV) could nearly completely inhibit productive viral replication (as assessed by p24 synthesis), even when added 12 hours after infection, and at 24 hours post-infection the drug still provided 30% of the maximal inhibition. Integrase inhibitors also showed significant inhibition up to 12 hours after infection (Vatakis et al., 2009). MLV vector transduction can also be inhibited by AZT (a nucleoside analog), but not HIV-targeted non-nucleoside reverse transcriptase inhibitors (NNRTIs). Lastly, as proteolytic maturation of both MLV and HIV vector particles occurs during or soon after release from the producer cell, protease inhibitors will not have any effect on transduction of a target cell. These results suggest that if an exposure occurred in a laboratory, carefully designed anti-retroviral therapy initiated immediately might reduce the number of cells transduced by the vector.

Lastly, an increased institutional awareness of the likelihood of such an accidental exposure, and the possibility that it might lead to harmful consequences, might lead to some pragmatic steps to allow attribution of causality should any laboratory workers develop cancer in the future. If vector transduction is causal in such an event, then the tumors will be marked with the integrat-
ed vector. To detect these vector DNA sequences, detailed structural information about the vector (i.e., an accurate DNA sequence and map) will be essential for the design of specific probes. Clear designation of the party responsible for storage of these sequences over the long-term would facilitate a determination of whether a retroviral transduction event was involved in tumor-igenesis or not.

In summary, the use of oncogene-containing retroviral vectors presents unique challenges with regard to biosafety of laboratory personnel. Although their replication-defective character eliminates the possibility that a viral infection could spread and infect the population at large, it is probable that at least some of these reagents possess the potential to enhance tumorigenesis after direct exposure. In that regard, they might be considered easily inactivated but very potent carcinogens. Relatively simple precautionary measures should significantly reduce the risk to laboratory personnel working with these reagents.

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International Biosafety and Biosecurity Challenges: Suggestions for Developing Sustainable Capacity in Low-resource Countries

Robert A. Heckert1, J. Craig Reed2, Felix K. Gmuender3, Maureen Ellis4, and Willy Tonui5

1Robert Heckert Consulting, LLC, Bowie, Maryland; 2Inspirion Biosciences, Frederick, Maryland; 3Basler & Hofmann Singapore Pte Ltd., Singapore; 4International Federation of Biosafety Associations, Ottawa, Ontario, Canada; and 5Kenya Medical Research Institute, Nairobi, Kenya

Abstract

The increased global demand for improved disease detection and control has resulted in the expansion of diagnostic and research capacity. However, the increase in infectious disease detection capacity has not necessarily been paralleled by an increase in biosafety and biosecurity capacity, particularly in low-resource countries. Low-resource countries face numerous challenges that severely constrain the development, or expansion, of sustainable capacity in biosafety and biosecurity management. This article divides these challenges into nine broad categories: 1) Country-/Region-specific Regulatory Framework and Guidelines or Standards; 2) Biosafety Awareness; 3) Infrastructure; 4) Equipment, Reagents, and Services; 5) Management Processes and Administrative Controls; 6) Biosafety Curricula; 7) Training; 8) Biosafety Associations, Professional Competency, and Credentialing; and 9) Individual Mentoring and Organizational Twinning.

Overcoming these challenges requires the collaborative efforts of representatives from the highest levels of local governments, the international biosafety community (e.g., international, regional, and national biosafety associations), and international development partners (e.g., national government agencies and programs, World Health Organization (WHO), World Bank, Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE)) to identify, fund, and execute solutions for sustainable capacity development. Collaboration is required to develop solutions appropriate for the specific needs and available resources within any given country.

Introduction

In the last decade a number of emergent, or re-emergent, diseases have reminded us of the constant threat posed by infectious diseases. These diseases affect humans, animals, plants, or multiple species, as recently demonstrated by the occurrence of zoonotic diseases, such as Severe Acute Respiratory Syndrome (SARS), avian influenza (H5N1), and the more recent influenza variant, H1N1. In each case, the rapid global spread of these diseases created enormous pressure on governments to diagnose, treat, and control the outbreaks. Thus, governments and international agencies were forced to examine their diagnostic, treatment, and research capacity for infectious diseases (Chan, 2010). In many low-resource countries, laboratory capacity was found to be inadequate and facilities poorly maintained (Kruk, 2008; The PLoS Medicine Editors, 2007; Wertheim, 2010; World Health Assembly, 2005). These findings generated increased interest from local governments, international agencies, and development partners to improve diagnostic, treatment, and research capabilities for both human and animal health in low-resource countries. The revised International Health Regulations (IHR) (WHO, 2005) call for all member states to rapidly detect, prevent, and respond to human and animal disease outbreaks. The regulations provide a framework to mobilize technical support and material resources in response to any disease pandemic. Laboratory biosafety and biosecurity are explicitly identified as critical components of IHR Core Capacity 8: Laboratory (www.who.int/ihr/IHR_Monitoring_Framework_Checklist_and_Indicators.pdf). This core capacity relates to laboratory services at each phase of infectious disease alert and response including detection, investigation and response, sample analysis performed in domestic laboratories, and shipment of specimens to collaborating centers. To fully and successfully reach this prescribed capacity, biosafety procedures must be improved or supplemented and regularly monitored, and laboratory bio-risk assessments must be conducted to guide and update biosafety regulations, practices, and procedures. While the requirement to identify and control disease outbreaks has increased diagnostic capacity (i.e., more facilities, more testing, more agent isolations), unfortunately, no corresponding increase in biosafety and biosecurity capacity has occurred. This is especially true in countries with limited resources.

Any country operating laboratories that provide some combination of diagnostic, clinical, and/or research activities involving human and/or animal pathogens should have laboratory and vivaria biosafety and biosecurity capabilities. And while all countries need to operate such laboratories, not all countries have the ability or resources to apply appropriate biosafety and biosecurity principles and practices to laboratory opera-
tions. In fact, biosafety and biosecurity are most compromised in low-resource countries where they are constrained due to numerous challenges. These challenges can be grouped into nine broad categories: 1) Country-/Region-specific Regulatory Framework and Guidelines or Standards; 2) Biosafety Awareness; 3) Infrastructure; 4) Equipment, Reagents, and Services; 5) Management Processes and Administrative Controls; 6) Biosafety Curricula; 7) Training; 8) Biosafety Associations, Professional Competency, and Credentialing; and 9) Individual Mentoring and Organizational Twinning. It is important to note that the challenges to biosafety management identified in these categories are not unique to low-resource countries; many are common to high-resource countries, as well.

“How best to address the spread of infectious diseases and to ensure the safe handling and secure storage of pathogens in laboratories is a matter of much debate. Gaining consensus is complicated by the fact that solutions that work for one nation may not work well for others due to varying funding priorities, limited resources, absences of regulatory framework, and sometimes ignorance of the risks” (Reed, 2010). This article identifies specific challenges in each of the nine categories listed above and provides recommendations to the end-user as well as to the international funding community.

The international community of supporters consists of many organizations and groups, including government development agencies, non-governmental aid agencies, large international bodies with global mandates, and individual endowments and trusts. While substantial investment will be required from international development partners, the funding of equipment, reagents, training, and minor facility improvements alone is inadequate to successfully overcome the existing challenges. Low-resource countries and development partners should first assess the full range of country-specific challenges across all nine categories and prioritize their goals against the funds available. This assessment process serves to ensure well-intended support funds are not applied in piecemeal or ineffective fashion. The essential final step is for the development partners and in-country leadership to mutually develop and adhere to a sustainability plan. A successful sustainability plan should define the time-phased transition of all direct and indirect program costs from the funder to the recipient to ensure that program objectives continue to be met in a self-sustainable manner following the depletion of donor funds.

For the purpose of this article, “low-resource” countries are defined as those countries possessing a Human Development Index (HDI) of 0.698 or lower in the 2011 United Nations (UN) Human Development Report (http://hdr.undp.org/en/statistics/). Countries with a HDI of 0.698 or lower include 94 of 187 countries. Use of the HDI is not meant to indicate that all countries below HDI of 0.698 suffer from an absence of biosafety or biosecurity capacity, rather, this index is referenced simply as a means of defining “low-resource” countries in the absence of a more appropriate quantitative measure or definition for the term.

**International Biosafety and Biosecurity Challenges**

**Country-/Region-specific Regulatory Framework and Guidelines or Standards**

Establishing and implementing regulatory guidelines or a framework for biological safety, complete with legislation and regulators to oversee implementation is essential for the consistent and uniform application of practices across all containment levels of a country’s laboratories and vivaria. Unfortunately, the majority of low-resource countries do not possess a regulatory framework for biosafety and biosecurity management and/or do not consistently adhere to, or formally endorse, the use of any particular guidelines for biosafety or biosecurity.

In establishing national biosafety and biosecurity guidelines, country officials may choose either to adopt or to adapt biosafety and biosecurity guidelines that have been developed by international organizations or other countries. The use of existing documents provides a solid foundation for establishing local biosafety guidelines and leverages existing resources. This approach should be attempted and promoted in countries that do not currently possess a written code of practice (Chua, 2009). The adaptation and tailoring of existing foreign country or international guidelines to suit the needs of any specific low-resource country are efforts best led by the appropriate Ministry(s) with technical support and input from representatives of national biosafety and other scientific professional associations (if present).

A given country can proceed to create guidelines or standards uniquely suited to its circumstances. The development community should support such actions as a means of establishing a sense of “ownership” among those professionals who will abide by the guideline as a result of having tailored biosafety and biosecurity practices to fit the regulatory oversight, cultural, and material constraints specific to the country. Consultation with existing international regulatory frameworks and stakeholders is imperative throughout the process to ensure compliance with international human and animal health regulations. Careful, country-wide risk assessments must be considered when contemplating the modification of internationally accepted norms; the reason for any deviation must be well substantiated and documented. For maximum benefit, final regulations or standards must be based upon sound principles of biosafety and biosecurity; they must be both mandated and endorsed at the national level.
In some instances, the absence of formal endorsement by the appropriate government ministries means that adopted or adapted guidelines are randomly and inconsistently applied thus generating an inconsistent approach to biosafety and biosecurity. Often this is due to a country’s lack of expertise and resources to implement, modify, or manage these guidelines or regulations. In some cases oversight functions are not present and in instances where oversight does exist, government inspectors may not possess the appropriate technical background and/or expertise to oversee the implementation of biosafety or biosecurity regulations. International funders should provide resources in the form of training to overcome these challenges.

**Biosafety and Biosecurity Awareness**

It is critical for those in positions of power and those who control budgets (e.g., laboratory directors and ministers to which these laboratories report, and elected officials) as well as laboratory scientists and facility managers to have a broad awareness of the importance of biosafety and biosecurity best practices. For international biosafety and biosecurity capacity development initiatives to succeed, the highest levels of government must formally endorse the principles and practices of biosafety and biosecurity risk management. Further, a commensurate allocation of financial resources must be available to support implementation of these programs.

Readily available biosafety and biosecurity technical literature is key to disseminating the principles and practices of biorisk management to the user community. Such literature would include: guidelines or standards published by the local government, other governments, or non-governmental organizations; textbooks; publications of biosafety societies; applied biosafety research articles; and instructional manuals. Each of these valuable instruments ensures the user community has a means to self-educate and remain current regarding progress and innovations in biosafety made by cohorts in other communities. National or regional biosafety associations can play an important role in disseminating and where applicable, translating this information in low-resource countries. The expanding use of computers and broad availability of the internet should result in greater ease of distribution and access to this information.

Due to a lack of biosafety information or knowledge of where information resides, senior leaders and decision makers sometimes lack a broad appreciation for the importance of biosafety and biosecurity best practices. Consequently, funding for biosafety and biosecurity is insufficient or essentially non-existent as these issues go unrecognized or are under prioritized. To complicate the situation, there is often a general absence of awareness of best practices at the laboratory level as well. In low-resource countries, development partners can help get local governments to commit to increased funding necessary to improve worker safety and pathogen security via awareness-raising activities including seminars and leave-behind informative literature targeted to elected officials, Ministry representatives, and laboratory senior management.

The general absence of awareness of best practices may be due, in part, to the dearth of subject matter content available in languages other than English. This lack of technical material printed in local languages is an important issue for non-English speaking scientists, technicians, and managers eager for this information. It also increases the challenges for Development partners that provide biosafety and biosecurity training. Therefore, existing national and international biosafety guidelines should be translated into those languages representative of low-resource countries. Development partners are often eager to support this type of activity since it results in a tangible product with a measurable impact.

To overcome the lack of technical guidance materials available in languages other than English, low-resource countries should either translate these materials locally or approach the organizations and agencies that developed the documents and request translated materials. To ensure accuracy, development partners should encourage bilingual subject matter experts in biosafety in these countries to review the translated materials prior to publication. When necessary, international development partners could help fund the cost of translation. Translated materials should receive the broadest distribution possible via postings on multiple websites, including that of the development partner, the appropriate country Ministry(s), as well as the national, regional, and international biosafety associations.

Scientists and laboratory directors in low-resource countries are responsible for improving biosafety awareness at the local level. These professionals can proactively share information among their colleagues and discuss policy solutions as issues arise. To attract funding for biosafety and biosecurity improvements, scientists and laboratory directors should actively seek collaborative relationships with organizations in more developed countries with robust biosafety and biosecurity capacities by making specific inquiries to academic, medical, and corporate enterprises. Various government agencies and programs, the International Federation of Biosafety Associations (IFBA), and other members of the international funding community are well positioned to facilitate these relationships, potentially fund collaborative projects, and establish twinning arrangements.

**Infrastructure**

Low-resource countries generally suffer a range of significant challenges associated with a lack of national infrastructure (e.g., absence of functional water purification and intact distribution systems, reliable electrical power, quality roadways and transportation networks,
adequate codes for construction and fire safety). While these challenges are beyond the scope of this article, these potentially overwhelming problems can negatively impact how containment laboratories and vivaria operate. Few members of the development community are in a position to address these challenges and such efforts are typically not well integrated across funding organizations. Decisions, actions, and funding to address national-level infrastructure problems often remain the responsibility of the low-resource country as its part of the in-kind contribution to projects.

In addition to national infrastructure, laboratory infrastructure has a direct and immediate impact on biosafety and biosecurity, as well. Existing laboratories in low-resource countries may suffer from a myriad of infrastructure problems typically associated with poor-quality starting materials combined with inadequate systems maintenance. The root of the problem is that laboratory operation budgets are typically inadequate to support the full cost of infrastructure improvement, routine maintenance, utility expenses, supplies and staff compensation. Consequently, individual laboratories, and sometimes entire facilities, are temporarily closed to save money. Conversations with facility directors and ministers in many low-resource countries consistently reveal the same problem: Existing national budgets are inadequate to maintain or replace these systems. International development partners that wish to help resolve these issues should approach the situation cautiously and with the awareness that the solution to any single issue can be costly and, arguably, futile. Development partners will be limited in how much they can enhance biosafety and biosecurity in low-resource countries unless all of the coexisting infrastructure challenges are identified and resolved simultaneously. While a comprehensive assessment of all facility systems is advisable prior to initiating improvements, this audit may show that it is less expensive and time-consuming to simply construct a new laboratory. This approach also has its challenges, as discussed below.

In developed countries, biocontainment facility design, construction, and operation have proven to be a very expensive endeavor. As the containment level of the laboratory increases, so does the cost and complexity of its systems. Modern high-containment laboratories require expert architectural design, specialized materials, careful workmanship from highly skilled workers, and technologically advanced systems engineering controls. The construction must conform to local codes. These high-biocontainment facilities operate with a high energy requirement, expensive technology, automated systems, safety equipment, and personal protective equipment. In low-resource countries numerous challenges are associated with acquiring and maintaining the containment laboratory infrastructure. These may include: the absence of laboratory design requirements or standards applicable for use in local settings; the inability to develop “design intent” documentation for use by the architectural and engineering firm; minimal input from end-users; too few appropriately skilled and experienced construction workers; absence of commissioning expertise; limited (if any) routine preventative maintenance; and a lack of experienced and full-time facility engineers. Although recommendations for each of these challenges are presented below, it is essential for representatives of low-resource countries to consider, pre-construction, the actual requirement for moving beyond biosafety level 2 containment laboratories and to recognize their country’s financial capability to sustainably operate and maintain high- or maximum-containment laboratories or vivaria.

If it is determined that constructing a high-containment facility is required, designers should take into account the country- or region-specific needs, capabilities, and resources. Unfortunately, none of the few laboratory construction guidance documents addresses laboratory or vivarium construction in low-resource countries (Mani et al., 2006; NIH, 2008; USDA, 2002). Consequently, it would be helpful for international funding partners or biosecurity associations to engage architects and engineers to develop options for low-resource countries and to support the translation of these new publications into multiple languages. Technical experts in national, regional, and international biosecurity associations could additionally work directly with architectural and engineering firms, local and major international construction companies, and facility systems manufacturers to help these firms focus on the unique end-user needs in low-resource countries. The associations and technical experts should identify reasonable options for laboratory design and construction involving lower cost, locally available materials and construction processes that involve less technical sophistication and specialized workmanship. The engineering solutions should be local, practical and sustainable (J. Welch, personal communication).

One of the most challenging aspects of building a containment facility is gaining a clear understanding of the performance requirements, or design intent, for the facility from the end-user. Developing a design intent document is crucial to enable an architectural and engineering firm to provide the end-user with a suitable facility. Challenges associated with creating the design intent document can be overcome to some extent through consultation with appropriately qualified technical experts within national or regional biosecurity associations.

Design problems notwithstanding, very few capable and experienced containment facility construction companies exist in most low-resource countries. Laboratories and vivaria, particularly those at biosafety level 3 and above, require specialized construction skills, materials, and workmanship. Unfortunately, there are too many examples of non-operational facilities and those that cannot be operated safely due to design and/or con-
struction failure. International funding partners should insist upon using an experienced commissioning team to prevent, identify, and address flaws through all phases of laboratory design and construction. In the rare cases where local commissioning standards do exist in a low-resource country, it is still difficult, if not impossible, to find local, knowledgeable, and experienced commissioning companies familiar with these standards. In these circumstances, outside experts will be necessary. Through raising awareness of the correct process and with international partner support, it is possible to complete the commissioning process and build well-constructed containment facilities.

Construction and commissioning challenges are just one hurdle to the development and expansion of biosafety and biosecurity capacity in low-resource countries. Another barrier is poor maintenance. It has been estimated that the annual maintenance budget for high-containment laboratories is often 10%-15% of the facility construction cost. Maintenance budgets are often overlooked during the initial facility planning, design, and budgeting process with few resources allocated for long-term maintenance, creating a false sense of confidence in the containment provided by the building systems.

Containment facilities require full-time, dedicated engineering staff to continually maintain the facility’s unique systems and equipment. It is crucial for successful facility operations to include these labor and equipment preservation costs in the operations and maintenance budget. In most low-resource countries, finding facilities that employ full-time engineers is rare and engineers are brought in as local tradesmen only when problems arise. In other cases, maintenance is not even recognized as a task requiring permanent staff, and instead is re-tendered annually or biannually. As maintenance costs are considered a burden, service contracts are typically awarded to the lowest bidder; this may result in poor facility and equipment performance.

Increasing awareness among the highest level government officials regarding the importance of laboratory and vivaria design, construction, operations, and maintenance is the best way to overcome laboratory infrastructure challenges and ensure ongoing availability of appropriate technical expertise. Additionally, development partners need to gain long-term commitment from the recipient partner that future budgets will cover projected operations and maintenance costs. Design, construction, and commissioning efforts must involve a mix of technically qualified expatriate labor matched with local nationals who will learn from the experience and remain in-country as a resource post-construction.

**Equipment, Reagents, and Services**

Operating clinical, diagnostic, and research laboratories requires a myriad of laboratory equipment and consumable reagents. In low-resource countries, the purchase and use of equipment, reagents, and support services present major challenges for the laboratories and the supporting development partners. As detailed below, these challenges include: availability of qualified individuals to inspect, maintain, repair, and certify specialized equipment items; unavailability of equipment, reagents, and services outside of capital cities; prohibitive costs for equipment, reagents, and services; over-engineered safety equipment that is too complex and energy-intensive for use in low-resource countries; excessive transport costs for heavy or temperature-sensitive items; and disposal problems with personal protective equipment (PPE) and laboratory materials.

Lack of availability of equipment, reagents, and support services presents a major challenge to biosafety and biosecurity capacity in low-resource countries. While a full range of these resources may be obtainable in select capital cities, they are often not available in less accessible areas. Until these materials are accessible through the local or national economy, biosafety program objectives will remain unsustainable. Development partners should help low-resource countries create and foster a business environment in which equipment manufacturers, distributors, reagent producers, and scientific service providers can compete and flourish. This will require conversations with senior government officials.

Some of the most important pieces of safety equipment operating in a containment laboratory are containment barriers. The standard example of this is the biological safety cabinet (BSC). Unfortunately, BSCs are underused, or simply not available, when they should be used according to best practices. A study by Astuto-Gribble et al. (2000) found that BSCs are used by only 64% of researchers who study pathogens that pose an inhalational hazard. Due to their importance in supporting biosafety in the laboratory and vivarium, funding partners would like to see more BSCs used. Unfortunately, in many parts of the world, funders cannot easily purchase BSCs locally, so they purchase them from U.S., Western European or Asian firms and ship them to the end-user facility. Given the size and weight of a BSC, transportation fees can equal the purchase price—effectively doubling the cost of each unit. Local experts to maintain, repair, or certify BSCs are similarly scarce and are often flown in at great cost to the institution. Working with infectious agents in a BSC that has not been properly positioned in the lab and certified is potentially more dangerous than working on the open bench. The creation of a business environment that allows equipment manufacturers, distributors, and scientific service providers to flourish would help to neutralize this challenge.

Development partners, as well as national, regional, and international biosafety associations, should work with equipment manufacturers to identify product characteristics and functions that meet the needs of scien-
tists in low-resource countries. For example, BSCs could be redesigned to be lighter weight and locally manufactured (i.e., constructed by less skilled craftsmen using readily available materials) with lower power requirements and clearly marked visual indicators that tell the operator the cabinet is operating within performance specifications. Since manufacturers are likely to find more sales at the bottom of the pyramid than at the top (Prahallad, 2009), information obtained from end-users should be used to frugally engineer the equipment (Sehgal et al., 2010).

Another potential solution is to rely less on modern safety equipment to reduce risk, and instead combine a thorough risk assessment with procedural modifications. These modifications should include procedures to reduce the generation of potentially infectious aerosols, encourage strong microbiological technique, and the use of appropriate PPE, such as respiratory protection, when recommended by the risk assessment. While this approach does not eliminate the requirement for BSCs in all situations, it may provide a reasonable alternative without compromising worker safety.

**Management Processes and Administrative Controls**

Practical organizational structures with effective management processes are critical components of a responsible biosafety program. Successfully integrating biosafety throughout an entire facility requires the presence of a dedicated Biosafety Director/Officer with the authority to develop and implement a comprehensive biosafety program. The system works best when all parties recognize the institutional authority of the Biosafety Director, complete their individual assignments willingly, and remain fully engaged in the compliance process.

Even though the international biosafety community values the principles and practices of biosafety and biosecurity, senior managers in low-resource countries may not prioritize, or endorse, the managerial and administrative processes required to implement such practices. In many countries organizational structures frequently lack a safety department, a Biosafety Officer, a designated Institutional Biosafety Committee, an Institutional Animal Care and Use Committee, and/or an Ethical Review Committee. Therefore, scientists and technicians attempting to implement sound biosafety practices at the bench will continually struggle in the absence of biosafety and biosecurity support at the top of the organization.

The European Committee for Standardization (CEN) Workshop Agreement (CWA) 15793:2008 (European Committee for Standardization, 2008) is a powerful tool that permits organizations to conduct self-assessments and identify those management processes and administrative controls needed to ensure effective biosafety and biosecurity implementation. Heightened awareness, translating internationally accepted technical documents into local languages, and providing international support in applying the management standard CWA 15793:2008 could go a long way in solving the issues related to the lack of administrative controls.

**Biosafety Curricula**

In many countries there is a demonstrated lack of understanding about the concepts of biosafety and biosecurity among scientists, laboratory managers, institute directors, and elected officials. Training biosafety practitioners, scientists, and management personnel would help address a portion of this problem. More important, safety and biosafety at the workplace should be integrated into and modeled in undergraduate and graduate laboratory courses. Instilling these principles into future biosafety professionals and scientists before they enter the workforce would help establish an integrated culture of safety in containment facilities. Ideally, professional biosafety associations, working with colleges and universities, would design and offer formal biosafety modules as a proactive way to shift from passive in-lab training to active instruction for the next generation of scientists and biosafety professionals. This approach would reach students at a formative period in their professional training and help ensure biosafety and biosecurity best practices become part of the backdrop of these individuals’ future work routines.

The national and private biomedical research funding organizations in each country should require that any scientific staff receiving grant support participate in annual biosafety refresher training as well as demonstrate competency in biosafety best practices related to their area of work. The international funding community should subsidize these initiatives as well as endorse the current IFBA initiative to create and implement a biosafety and biosecurity curriculum that culminates in an internationally recognized diploma.

**Training**

Training is a highly effective means of changing norms and paradigms. Developing expert training staff via a Train-the-Trainer model is an efficient way to initiate and maintain long-term change in biosafety practices in low-resource countries or regions. A national or regional biosafety association can provide leadership by identifying individuals qualified to become the next generation of trainers within each country. Unfortunately, this process has been hampered by the lack of training materials in the appropriate language that can be provided to the local trainers and left In-country as a resource.

The WHO, various other government agencies, and a very few private-sector organizations provide biosafety and biosecurity support and training throughout the world. Their efforts would be exponentially enhanced if the training materials developed for these efforts were consolidated and more widely used through the
national, regional, and international biosafety associations. Regardless of the source, any training program needs comprehensive buy-in from the local or regional biosafety associations. For English-speaking countries, a wealth of free biosafety and biosecurity training materials is available on the web sites of the IFBA (www.internationalbiosafety.org) and the American Biological Safety Association (ABSA) (www.absa.org).

**Biosafety Associations, Professional Competency, and Credentialing**

A biosafety association is a non-profit, apolitical group of like-minded professionals committed to sharing resources, experiences, and problem-solving approaches for their mutual benefit and furthering knowledge in biosafety and biosecurity. Biosafety associations are intended to support the biosafety and biosecurity objectives of individuals as well as government, commercial, academic, and private organizations. These associations play a critical role in disseminating information to their constituent members while simultaneously presenting members’ knowledge to government decision makers formulating guidelines and policy. Biosafety associations exist at the local, state, national, regional, and global levels. Some examples of long-standing national associations include ABSA, the Canadian Association of Biological Safety, and the Biosafety and Biosecurity Association of Brazil; regional biosafety associations include the Asia-Pacific Biosafety Association (A-PBA) and the European Biological Safety Association—each of which separately represents over 20 different countries in its region. IFBA, a global biosafety organization, is currently composed of 17 different national and regional member organizations that collectively represent over 120 different countries.

National biosafety associations are rare in low-resource countries simply because of the lack of support and endorsement from national, academic, and corporate organizations. The development community should support the establishment of biosafety associations by actively communicating the purpose and mission of such organizations to local government officials. The international community can also support scientists and biosafety professionals working to establish local biosafety associations.

Securing adequate funding for the operation of a biosafety association can be challenging. Membership fees are sometimes viewed as a barrier to success for start-up biosafety associations to the extent these fees may discourage prospective members from joining. It is important to note, however, that the majority of revenue for the operation of a biosafety association often comes from the payment of registration fees to the annual conference and to pre-conference training courses and not from membership dues. Start-up biosafety associations could potentially identify corporate, non-government, or federal sponsors to support the organization’s operating costs, and work to reduce their operating costs in general, with the goal of lowering or eliminating membership fees in low-resource countries.

Two regional biosafety associations, the African Biosafety Association (AfBSA) and A-PBA, have worked together to form an outreach and seed program that fosters biosafety in their respective geographical regions. The program has proven remarkably successful and should serve as a model for marketing biosafety associations around the world. Annual conferences are held in different locations, thus increasing potential support across the region. In preparation for the annual conference, these regional associations reach out to the local microbiology, virology, and human/animal health professionals of the host country and help them to organize their own national biosafety association. With these actions, the regional biosafety associations are driving the creation of national biosafety associations. For example, the AfBSA and A-PBA provide financial and personnel support until the local committee can effectively operate its own association. This successful model proves biosafety associations can help one another further their shared purposes.

Another major challenge to building biosafety and biosecurity capacity in low-resource countries is the absence of a relevant biosafety credentialing system. Only the United States has a formal biosafety credentialing system. This system is administered by ABSA and two designations are available: Certified Biological Safety Professional (CBSP) (www.absa.org/biocert.html) and Registered Biosafety Professional (RBP) (www.absa.org/bioreg.html). The cost of obtaining and maintaining these credentials is significant, if not impossible, financial burden for biosafety professionals in low-resource countries. To overcome these challenges, low-resource countries should support organizations (regional/national/global biosafety associations) in the development of credentialing requirements that are relevant and maintainable by biosafety professionals in the country or region.

**Individual Mentoring and Organizational Twinning**

Junior biosafety practitioners, career scientists, laboratory managers, and others new to the field of biosafety can benefit from mentoring. IFBA and ABSA administer a mentoring program that pairs individuals with mentors and takes cultural and geographical considerations into account. The mentor is expected to assist with technical questions, provide recommended readings about issues related to biosafety and biosecurity, and assist with professional introductions to other members in the field.

The institutional equivalent to mentoring is organizational twinning. Twinning allows young or developing biosafety associations to benefit from the knowledge and experience gained by existing national or regional
biosafety associations. These relationships increase the likelihood of success for the younger organization. For example, A-PBA currently serves as the organizational twin to the Biosafety Association of Central Asia and the Caucasus.

Summary and Conclusions

Creative solutions exist to make the seemingly insurmountable challenges, of implementing and expanding biosafety and biosecurity capacity in low-resource countries, very achievable tasks. The process begins by bringing together knowledgeable local professionals, in a variety of different leadership positions, who understand the risks associated with the manipulation, transport, and storage of pathogens. With the help of international funding partners, these individuals can identify gaps in their biosafety and biosecurity risk management and thoughtfully identify and assign country-specific resources to mitigate these risks. When assistance from the international funding community is requested, or even required, it is incumbent upon the low-resource country to carefully assign these resources for maximum value. Most important, responsible officials from the low-resource country must help these containment facilities become self-sufficient by creating a viable sustainability plan that transitions all program costs from the funder to the recipient over a defined period of time.

While supporting low-resource countries in the development and application of appropriate biosafety and biosecurity best practices is challenging, funding partners and low-resource countries need to acknowledge these challenges in order to work together to overcome them. As stated by Chua et al. (2009), “In our quest to provide a safer world today and for the generations to come, we must shift our consciousness from national responsibility to one of a global responsibility...from a national community to a global community.”

Acknowledgments

We thank our biosafety colleagues from around the world who have shared their experiences with us and contributed to the identification of the challenges and recommendations presented. We also thank Ms. Jennifer Cole, Frontline Healthcare Workers Safety Foundation, Ltd., for editorial contributions. *Correspondence should be addressed to Robert A. Heckert at rheckert@safivet.com.

References


Enhancement of the Mentored Training Program for Investigative Staff at the University of Pittsburgh Regional Biocontainment Laboratory

Lesley C. Homer*, Amy L. Hartman, Dennis T. Heflin, Anita M. Trichel, Douglas S. Reed, and Kelly Stefano Cole

University of Pittsburgh, Pittsburgh, Pennsylvania

Abstract

This article provides a description of the improvement and enhancement of the mentored training program for investigative staff at the University of Pittsburgh Regional Biocontainment Laboratory (UPitt RBL) as originally described in "Evolution of a facility-specific BSL-3 training program for the University of Pittsburgh Regional Biocontainment Laboratory" (Hartman, 2010). During the third year of operation of the UPitt RBL, the Team responsible for managing the facility identified a number of specific areas for improvement in the mentored training program, including internal communication among the Team and between the Team and investigators, verifying proficiency at biosafety levels 1 and 2 prior to initiating training at biosafety level 3, establishing mentor qualifications, clarifying and restructuring the training assignments for mentor and trainee, improving the training documentation, and requiring final observation by management prior to granting independent access. Upon completion of the objectives, the Team formalized the program and implemented it for all new investigative trainees. The enhanced program provides a higher level of assurance that lab personnel are competent and proficient to work with biosafety level 3 agents.

Background

Most biological safety officers would probably agree that training is a critical component of safe work in laboratories. But because training is a broad topic that may include classroom instruction, demonstration, and hands-on work under observation, it is likely that each biological safety officer has a unique opinion on how to train, what to train, and how to measure training effectiveness. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, includes a recommendation that lab personnel be trained appropriately and must "demonstrate proficiency in standard and special microbiological practices before working with BSL-3 agents" (U.S. Department of Health and Human Services, 2009). However, the BMBL does not provide guidance on how to train effectively to specific levels of proficiency or how exactly to measure proficiency (Chamberlain et al., 2009). One-on-one training (a.k.a., mentored training) and direct observation of good laboratory practices have been suggested as equally important if not more important than classroom-style training (Tun et al., 2009) and may provide a greater level of assurance of proficient performance in the lab or animal facility (Foshay et al., 2007).

Training may have value beyond demonstrating competency in the laboratory. Recently, both the National Science Advisory Board for Biosecurity (NSABB) and the Federal Experts Security Advisory Program (FESAP) examined practices in high-containment labs and each entity issued a series of recommendations to improve the security of biological agents (FESAP, 2011; NSABB, 2011). Both entities identified training and ongoing performance evaluations as important means to assess the suitability of lab personnel granted access to Select Agents. NSABB concluded that “Strong institutional and laboratory leadership, clear articulation of priorities and expectations, and an institutional framework that provides relevant education, training (emphasis added), performance review, and employee support will facilitate responsible practices, personnel reliability, safety, and security” (NSABB, 2011). A requirement for pre-hire and post-hire assessment of reliability and suitability for lab personnel given access to the highest-risk Select Agents is included in the recent proposed rule, although it does not include specific mechanisms for determining suitability, and the only significant change in the training requirement is to provide insider-threat training (Office of the Federal Register, 2011). Both NSABB and FESAP considered additional means to assess reliability and suitability, such as credit checks, criminal background checks, drug testing, review of credentials, and mental health assessments, with varying degrees of recommendation. These mechanisms are probably less accessible to biosafety officers and facility managers than training.

The UPitt RBL is a large, multi-user BSL-3 enhanced facility that contains approximately 20,000 square feet of BSL-3 containment space divided into three BSL-3 laboratory suites and four individual animal BSL-3 suites. Approximately 20 infectious agents, including Select Agents, (CFR 2008a, 2008b, 2008c) are stored or manipulated in the facility. Six animal species ranging from mice to non-human primates have been utilized for animal models of disease. Due to the size and complexity of the work conducted in this facility, the UPitt RBL
has a dedicated RBL Management Team ("the Team") consisting of an RBL Associate Director, an RBL Research Manager, an RBL Operations Manager/Associate Vice-Chancellor, an RBL Biosafety Officer (RBL BSO), an RBL Attending Veterinarian, an RBL Veterinary Supervisor, and an RBL Aerobiology Manager.

As discussed in the prior publication (Hartman, 2010), work with Select Agents and Risk Group (RG) 3 agents was limited to a few investigators at the University prior to the opening of the UPitt RBL, and there was no centralized training program for either type of work. The Team felt that designing a training program for all personnel listed on the RBL’s Select Agent registration was necessary to best prepare personnel for safe work in the facility. Training programs should be dynamic and evolve with changes such as scope of work or understanding of training needs, and so the Team continuously improved and refined the training program over the course of the first 2 years of the UPitt RBL operation. For at least 2 years, the Team required trainees to complete the following three phases of training:

- A BSL-3 classroom-based training course equaling 16 hours of education, primarily administered by the RBL BSO, consisting of 10 training modules such as BSL-3 practices and facility design, use of personal protective equipment, use of safety equipment, occupational health programs, and safe work with animals, as well as tabletop exercises and quizzes
- A half-day facility orientation administered by the RBL BSO to bridge the gap between the classroom training course and actual work in the UPitt RBL. This occurs only after the individual is approved for Select Agent Security Risk Assessment (SRA)
- A period of mentored training for new investigative employees to demonstrate proficiency at BSL-3, administered by various Management Team members as well as investigative staff deemed appropriate for mentorship.

After 2 years of operation, the Team had provided about 140 individuals with the RBL BSL-3 classroom-based training course. The Team had provided about 100 individuals with the half-day of RBL facility orientation, which was developed toward the end of the first year of operation, and the Team had provided mentored training to about 50 individuals. During the third year of operation, no new investigators joined the UPitt RBL, and the staffing of approved investigators, building engineers, and veterinary technicians stabilized. As a result, the rate of new employees entering the training pipeline slowed to approximately one new trainee per month.

At this point, the Team critically examined the mentored training program to determine if it provided the most robust means possible to ensure that trainees are competent and proficient prior to granting access to BSL-3 agents and Select Agents. The Team identified areas for improvement including goals, mentor qualifications, documentation, communication, and personnel approval.

**Objectives**

During the second and third years of operation, the typical process for investigative staff to undergo training for work in the UPitt RBL started with a request from the investigator to either the RBL Associate Director or the RBL BSO. The RBL BSO then scheduled the trainee for a fingerprinting session for the SRA, notified the trainee of the general training and medical surveillance requirements, and provided the trainee with the UPitt RBL training course materials. Upon SRA approval, completion of the UPitt RBL training course and verification of all training and occupational health prerequisites required by UPitt, the RBL BSO provided the trainee with the half-day orientation session. Based upon the trainee’s previous experience at BSL-3, the RBL BSO collaborated with the Research Manager to determine requirements for mentored training for each trainee. Various members of the Team and/or investigative staff provided the mentored training and reported results to the RBL BSO in the form of a training checklist listing the dates that mentored training was provided. Most trainees underwent five

**Figure 1**

Mentored training in a BSL-3 laboratory.
mentored training sessions. Figure 1 depicts a mentored training session in which a postdoctoral fellow provided training on bacterial culture techniques.

Upon evaluation of the original mentored training program, the Team identified the following objectives to improve the program:
• Improve internal communication within the Team and between the Team and investigators about the progress of trainees.
• Verify proficiency at BSL-2 prior to undergoing BSL-3 training.
• Clarify and restructure the training assignments within the Team.
• Establish mentor qualifications.
• Define the roles of mentor and trainee during sessions.
• Improve the training checklists.
• Require final observation by the Team prior to approval.

**Improve Communication**

The Team requested more frequent and more thorough internal communication about the progress of new trainees. As a result, the RBL BSO created a spreadsheet to track the training status of each new employee and his or her respective investigator. As shown in Table 1, the first mock trainee has completed all of the steps in the training pipeline and has received access to work independently in the facility. The second mock trainee has completed some but not all of the steps in the training pipeline.

On a monthly basis, the RBL BSO reviews the training spreadsheet and notifies the Team of any trainees who are nearing the completion of training and/or facility orientation. The RBL BSO documents the initiation of mentored training as well as the name of the mentor and the date of completion of the mentored training on the training spreadsheet. Once all mentored training has been completed, the RBL BSO presents these data to the Team and either requests approval for the individual to receive facility access and work independently in the UPitt RBL, or recommends further training for the individual. A unanimous decision by all Team members is required to approve a trainee. The date of approval and the date that access was granted to the BSL-3 facility are recorded on the training spreadsheet.

**Verify Proficiency at BSL-2**

The Team holds scheduled monthly meetings with each RBL investigator to review current and upcoming projects, provide safety and regulatory updates, and discuss other facility issues. Training and new trainees are also discussed. The Team sought to encourage investigators to provide more information about new trainees and to formally accept new trainees prior to the start of the approval and training process. As a result, each investigator now provides an explanation of the anticipated role of each new trainee along with a summary of the trainee’s experience in the laboratory and in the animal research environment. The RBL BSO determines the mentored training requirements and the investigator identifies the mentor and approves the training requirements. This is documented in the minutes of the monthly investigator meetings. The Team also felt that it is appropriate for the investigator to provide documentation that each new trainee is proficient at BSL-2. The Team now requires completion of a checklist for BSL-2 knowledge and proficiency (Tables 2 and 3) for each

**Table 1**

Data for mock trainees, representative of the training spreadsheet provided to the RBL Management Team on a monthly basis.

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Name</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Joan Smith (Brown lab)</td>
<td>Mike Jones (Jackson lab)</td>
</tr>
<tr>
<td>Submitted SRA</td>
<td>1/4/2011</td>
<td>12/10/2010</td>
</tr>
<tr>
<td>Received SRA approval</td>
<td>3/2/2011</td>
<td>3/15/2011</td>
</tr>
<tr>
<td>Started RBL BSL-3 training</td>
<td>2/15/2011</td>
<td>1/16/2011</td>
</tr>
<tr>
<td>Completed RBL BSL-3 training</td>
<td>3/15/2011</td>
<td>2/12/2011</td>
</tr>
<tr>
<td>Started mentored training</td>
<td>4/2/2011</td>
<td>5/15/2011</td>
</tr>
<tr>
<td>Completed mentored training</td>
<td>5/15/2011</td>
<td>Pending</td>
</tr>
<tr>
<td>Approved by Management Team</td>
<td>5/23/2011</td>
<td>Pending</td>
</tr>
<tr>
<td>Received access to BSL-3</td>
<td>5/23/2011</td>
<td>Pending</td>
</tr>
</tbody>
</table>
Table 2

Example section headings and tasks from the checklist for BSL-2 proficiency.

<table>
<thead>
<tr>
<th>Tasks: Biosafety Level 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perform hand washing after working with potentially hazardous agents &amp; before leaving the lab.</td>
</tr>
<tr>
<td>Refrain from eating or drinking in the lab.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Biosafety Level 2 (in addition to BSL-1 tasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport potentially infectious materials only in leak-proof labeled containers.</td>
</tr>
<tr>
<td>Perform all aerosol-producing procedures only inside BSC.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Use of a biological safety cabinet (BSC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verify that the BSC is current for annual certification and is safe to use.</td>
</tr>
<tr>
<td>Demonstrate how to turn the blower on and off, and where to place the sash.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Animal Biosafety Level 1 (in addition to BSL-1 tasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demonstrate safe handling of animals (grip techniques, positioning animal).</td>
</tr>
<tr>
<td>Demonstrate competence in applicable veterinary procedures such as blood collection, injection, other surgical procedures, and euthanasia and necropsy.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Animal Biosafety Level 2 (in addition to all above tasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demonstrate proper use of physical and chemical restraints to handle animals.</td>
</tr>
<tr>
<td>Physical restraints may include engineering controls such as squeeze mechanisms.</td>
</tr>
</tbody>
</table>

Table 3

Example section headings and concepts from the checklist for BSL-2 proficiency.

<table>
<thead>
<tr>
<th>Tasks: Biosafety Level 2 (in addition to BSL-1 tasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be aware of post-exposure procedures in case of infectious agent exposure.</td>
</tr>
<tr>
<td>Understand emergency response procedures in the lab.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Understand how infectious agents are transmitted in the research setting:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Direct skin, eye, or mucosal membrane exposure</td>
</tr>
<tr>
<td>• Sharp injury (needle stick, scalpel cut)</td>
</tr>
<tr>
<td>• Animal bite or scratch</td>
</tr>
<tr>
<td>• Inhalation of droplets or aerosols</td>
</tr>
<tr>
<td>• Ingestion due to accidental contamination of hands/food/drink</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Demonstrate familiarity with biological hazards:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Natural mode of infection</td>
</tr>
<tr>
<td>• Means of transmission in the lab</td>
</tr>
<tr>
<td>• Susceptibility to disinfectants and treatment methods</td>
</tr>
<tr>
<td>• Recommended lab safety practices</td>
</tr>
<tr>
<td>• Occupational health requirements</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Use of a biological safety cabinet (BSC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Understand the principles of a Class II BSC (HEPA-filtered air over the work surface, room air drawn in through the front grille, and HEPA-filtering of exhaust air).</td>
</tr>
<tr>
<td>Understand why the use of flames and gas burners should be avoided.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tasks: Animal Biosafety Level 2 (in addition to all above tasks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handle and disinfect bedding, carcasses, tissues, and cages</td>
</tr>
<tr>
<td>Demonstrate understanding and proper use of biocontainment caging.</td>
</tr>
</tbody>
</table>
new trainee prior to starting the mentored training program. The checklist is reflective of current standards (CDC, 2011; U.S. Department of Health and Human Services, 2009) and is divided into the following sections: BSL-1, BSL-2, use of a biological safety cabinet (BSC), understanding general practices for cell and tissue culture, animal BSL-1, and animal BSL-2. The checklist addresses specific tasks (Table 2) as well as general concepts (Table 3). The investigator or mentor completes a checklist for each prospective trainee and provides it to the RBL BSO at the start of mentored training.

**Clarify and Restructure the Training Assignments**

For the first year of the mentored training program, the Team made a standard assignment of three to five training sessions for trainees without any BSL-3 experience. A “session” was defined as one period of work inside the BSL-3 facility, including garb-in and garb-out/shower-out, typically to last approximately 1 hour. If a trainee intended to perform work with animals but had no animal BSL-3 (ABS-L3) experience, then the Team assigned an additional three to five training sessions with animals. These training sessions focused on basic safety procedures such as garbing into the suite, proper use of respiratory protection, use of a BSC, use of animal caging, waste decontamination and disposal, and proper exit procedure. The training sessions proved useful to help lab personnel understand how to conduct procedures in the BSL-3 facility that they had previously performed at BSL-2 only. For example, some lab personnel did not have experience loading tubes into centrifuge rotors in the BSC and did not have experience with centrifuge safety lids. The training sessions allowed lab personnel to practice these steps and to understand that these steps added time to the procedure. If a trainee had BSL-3 experience outside the UPitt RBL, then the Team typically reduced the mentored training sessions to one entry into the facility, under escort by the RBL BSO, to verify basic safety procedures were understood. The Team did not require investigators with extensive BSL-3 experience or employees who transferred from one UPitt RBL work area to another to undergo additional mentored training.

To enhance the mentored training program, the Team felt that assignment of training sessions should be standardized and that mentored training should never be waived. The Team revised the assignment of mentored training for new investigative staff (Table 4). The Team now requires a minimum of five documented laboratory training sessions for trainees with less than 6 months of prior BSL-3 experience. The Team now requires three documented laboratory training sessions for trainees with more than 6 months of prior BSL-3 experience. For individuals who transfer from one BSL-3 work area to another, the Team now considers the change in responsibility and then assigns mentored training as seems reasonable based upon the scope of work and the pathogen. Similar requirements are applicable for work with animals and are shown in Table 4. For individuals with no animal experience, the investigator must provide at least 3 months of BSL-1 or BSL-2 animal training to the trainee outside of the UPitt RBL before allowing mentored training to begin.

**Establish Mentor Qualifications**

To further enhance the mentored training program, the Team defined minimum mentor qualifications. The Team asserted that only senior, trusted, experienced personnel with full access to the UPitt RBL may serve as mentors (Le Duc et al., 2008). Tables 5 and 6 show the mentor qualification guidelines now in use for laboratory and animal facility training, respectively. In general, members of the Team may mentor any new trainees, although it is understood that Team members will focus on his or her area of expertise. Investigators and their senior staff with experience with the same or with closely related pathogens and/or animal species in use may mentor their own trainees and training needs for new pathogens and animal species with which the investigator has less experience are assessed on a case-by-case basis. Investigators are strongly encouraged to identify a single mentor to achieve consistency in practices among

<table>
<thead>
<tr>
<th>Area</th>
<th>Prior Experience</th>
<th>Mentored Training Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>&lt;6 months of BSL-3 experience</td>
<td>At least 5 training sessions</td>
</tr>
<tr>
<td>Laboratory</td>
<td>&gt;6 months of BSL-3 experience</td>
<td>At least 3 training sessions</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Transfer from one BSL-3 work area to another</td>
<td>Determined on a case-by-case basis</td>
</tr>
<tr>
<td>Animal</td>
<td>No animal experience</td>
<td>At least 3 months of non-BSL-3 animal training by PI prior to starting mentored training in RBL</td>
</tr>
<tr>
<td>Animal</td>
<td>&lt;6 months of ABS-L3 experience</td>
<td>At least 5 training sessions</td>
</tr>
<tr>
<td>Animal</td>
<td>&gt;6 months of ABS-L3 experience</td>
<td>At least 3 training sessions</td>
</tr>
<tr>
<td>Animal</td>
<td>Transfer from one ABS-L3 work area to another</td>
<td>Determined on a case-by-case basis</td>
</tr>
</tbody>
</table>
trainees. All mentors must have documented qualifications such as curriculum vitae on file with the RBL BSO. The Team restricted graduate students from serving as mentors as they are focused on study and original research and the Team does not consider them as senior research staff.

**Define Roles**
For the first year of the mentored training program, the RBL BSO led at least one of the training sessions and focused this session on basic safety procedures for BSL-3. Training sessions led by the Research Manager focused on assisting new personnel in translating projects from BSL-2 to BSL-3. For training sessions led by the mentor, the mentor did not usually plan or document any specific experiments for the sessions in which the trainee observed the mentor performing work or in the sessions in which the mentor observed the trainee performing work. The Team determined that mentored training is best accomplished when the mentor observed the

### Table 5
Mentor qualification guidelines for BSL-3 laboratory training.

<table>
<thead>
<tr>
<th>Mentor Job Title</th>
<th>Minimum Requirements</th>
<th>Approved Mentorship Responsibilities</th>
<th>Documentation Required (i.e., CV on file)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBL Management Team member</td>
<td>1 year of experience working at BSL-3 or managing a BSL-3 facility; 6 months working in or managing the UPitt RBL</td>
<td>All personnel</td>
<td>Yes</td>
</tr>
<tr>
<td>Principal Investigator</td>
<td>1 year of experience working at BSL-3; 1 year of working in the UPitt RBL. At least 1 year of experience with the pathogens in use in the RBL</td>
<td>His/her direct reports</td>
<td>Yes</td>
</tr>
<tr>
<td>PI Postdoc or Senior Technician</td>
<td>B.S. in biology or related life sciences field; 1 year of experience working at BSL-3; 1 year working in the UPitt RBL; At least 1 year of experience with the pathogens in use in the RBL</td>
<td>Peers (reporting to same PI)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Table 6
Mentor qualification guidelines for BSL-3 animal training.

<table>
<thead>
<tr>
<th>Mentor Job Title</th>
<th>Minimum Requirements</th>
<th>Approved Mentorship Responsibilities</th>
<th>Documentation Required (i.e., CV on file)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBL Management Team Member</td>
<td>1 year of experience working at BSL-3; 6 months working in or managing the UPitt RBL</td>
<td>All personnel</td>
<td>Yes</td>
</tr>
<tr>
<td>RBL Vet and Husbandry Technicians</td>
<td>1 year of experience working with animals in BSL-3; 1 year working with animals in the UPitt RBL</td>
<td>All personnel; Animal species and husbandry procedures with which he/she has experience</td>
<td>Yes</td>
</tr>
<tr>
<td>Principal Investigator</td>
<td>1 year of experience working with animals in BSL-3; 1 year working with animals in the UPitt RBL</td>
<td>His/her direct reports; Animal species, pathogens, and procedures with which he/she has experience</td>
<td>Yes</td>
</tr>
<tr>
<td>PI Postdoc or Senior Technician</td>
<td>B.S. in biology or related life sciences field; 1 year of experience working with animals in BSL-3; 1 year working with animals in the UPitt RBL</td>
<td>Peers (reporting to the same PI)</td>
<td>Yes</td>
</tr>
</tbody>
</table>
trainee performing work; therefore, guidelines were established for the roles of mentor and trainee. To begin, the mentor performs work with the trainee acting as observer at least once. The mentor may choose to perform work under the observation of the trainee for as many sessions as he or she deems appropriate, but the Team credits this only as one mentored training session. The majority of training should be accomplished by the trainee performing work under the observation of the mentor inside the BSL-3 facility. This is communicated to the mentor and trainee prior to the start of training and is documented in the training checklist for each session. A final and separate observation by the Team completes the mentored training (see below).

**Improve the Training Checklists**

The Team wrote laboratory and animal facility training checklists and provided them to the investigators and mentors; however, the mentors did not consistently use them, sometimes substituting a handwritten note or a table of entry dates and times, or sometimes combining multiple sessions onto one checklist. The Team wanted the standard checklists to be used for each training session and wanted a separate training record for each session. Table 7 lists the section headings in the revised checklists for laboratory and animal training. On each checklist, the mentor records the names of trainee and mentor, the date, the biological agents in use, the procedures performed within each section, and who performed each procedure. Both the trainee and the mentor initial each applicable task that is performed (or each concept that is discussed) during the training session. Space is given at the end of the checklist for the mentor to annotate any areas for improvement. The mentor then faxes the checklist to the RBL BSO. Because animal procedures may vary widely from project to project or even species to species, many optional tasks (Table 8) or fill-in-the-blank areas are part of the animal facility checklist. A separate checklist must be provided for each session for the session to count toward fulfillment of training.

### Table 7

Section headings in the revised checklists for BSL-3 laboratory and animal training.

<table>
<thead>
<tr>
<th>BSL-3 Laboratory Training</th>
<th>BSL-3 Animal Training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry into the BSL-3 facility</td>
<td>Entry into the ABSL-3 facility</td>
</tr>
<tr>
<td>Use of a biological safety cabinet</td>
<td>Use of a biological safety cabinet</td>
</tr>
<tr>
<td>Use of other lab equipment</td>
<td>Animal procedures</td>
</tr>
<tr>
<td>Select Agent inventory</td>
<td>Animal transport</td>
</tr>
<tr>
<td>Disinfection and waste disposal</td>
<td>Use of other animal equipment</td>
</tr>
<tr>
<td>Exit from the BSL-3 suite</td>
<td>Disinfection and waste disposal</td>
</tr>
<tr>
<td>Exit from the BSL-3 facility</td>
<td>Exit from the ABSL-3 facility</td>
</tr>
</tbody>
</table>

### Table 8

Some of the optional tasks in the revised checklist for BSL-3 animal training.

<table>
<thead>
<tr>
<th>Task Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>If containment cage is in use, verify the containment cage is working properly.</td>
</tr>
<tr>
<td>Demonstrate proper use of additional protective equipment required to handle infectious animals.</td>
</tr>
<tr>
<td>Remove animal from its cage and transport animal to work area. Annotate physical or chemical restraints used.</td>
</tr>
<tr>
<td>Demonstrate safe handling of animals (grip techniques, positioning animal). Annotate physical or chemical restraints used.</td>
</tr>
<tr>
<td>Demonstrate competence in applicable veterinary procedures. (Check/annotate all that apply.) Injection Phlebotomy Nasal wash Ear tag punch Bronchialveolar lavage Ear bleed Other (specify)</td>
</tr>
<tr>
<td>Demonstrate competence in euthanasia and necropsy: Euthanasia (specify method) Necropsy</td>
</tr>
<tr>
<td>Use of other equipment in the ABSL-3: X-ray Endoscope Necropsy table Mobile transfer cart Other (specify)</td>
</tr>
</tbody>
</table>
Require Final Observation by Management Team

As stated previously, the Team felt a responsibility to increase its role in the oversight of mentored training. The Team decided that one Team member must perform a final observation of each new trainee upon completion of mentored training and that this should fall under the responsibility of the RBL BSO in most cases. The RBL BSO typically contacts the mentor to determine an appropriate procedure for the trainee to perform. The RBL BSO observes the trainee entering the facility including sign-in to locker room, clothing change, use of wireless communication badge, entry into the suite including donning protective equipment, performing the identified procedure, use of the biosafety cabinet and other laboratory equipment, performing decontamination and waste disposal, exit from the suite including removing protective equipment, and exit from the facility including sign-out of the locker room. The RBL BSO observes the trainee to ensure that he or she appears comfortable with and confident in the general entry/exit procedures and the specific laboratory procedures. If during final observation the RBL BSO deems the training satisfactory, then the trainee may receive access to work in the suite including donning appropriate BSL-3 suit(s). If the RBL BSO deems the training unsatisfactory, then the investigator is consulted and further training is likely to be required.

Refresher Training

All individuals with access to the UPitt RBL are required to attend annual refresher training on biosafety, security, and incident response. The Team requires supplemental training when there are significant changes to the biosafety, security, or incident plans, and refresher training including entry into at least one BSL-3 suite is required for individuals who have not worked in the RBL for more than 6 months and who wish to retain access.

Case Study

Following the revision of the mentored training program, each investigator was provided with information on the revised program as well as the new checklists. At a subsequent meeting between the Team and an investigator, the investigator reported that a trainee was ready to begin the mentored training program. This investigator studied virus-cell interaction using alphaviruses and flaviviruses including two Select Agents. The mouse model was used. A postdoctoral fellow was trained to perform all procedures in the UPitt RBL and had been designated as the mentor for all of the investigator’s trainees. This trainee was a graduate student under the advisement of the investigator and had received his SRA approval, completed the UPitt RBL BSL-3 training course, and completed all general occupational health and training requirements. The trainee had worked for over one year in a BSL-2 laboratory and had several months of experience with mice infected with agents categorized as RG2. The Team asked the investigator and his designated mentor (the postdoctoral fellow) to submit the BSL-2 proficiency checklist and a revised BSL-3 or ABSL-3 checklist for each training session. This was a change for the investigator as he typically allowed his mentor to simply track the dates and times of training for each new trainee in a table and then to submit the table to the RBL BSO. Once the Team explained the rationale for additional documentation requirements to the investigator, he agreed to a requirement of five laboratory training sessions and five animal facility training sessions.

The RBL BSO contacted the trainee to schedule the orientation session. Upon completion of the orientation session and receipt of the BSL-2 checklist, the RBL BSO notified the mentor that mentored training could begin. Over the course of 4 weeks, the mentor completed a checklist for each training session with the trainee, covering tasks such as growing and harvesting viral stocks, producing and testing virus-based systems for lack of propagation competency, isolating nucleic acids, monitoring infected mice for disease development, euthanasia, and necropsy. During this period of time, the Team revised the checklists to require that the mentor must list the procedures performed and must specify who performed each procedure. The Team required this change in order to document that the majority of training sessions consisted of the trainee performing work under the observation of the mentor as described in the section “Define Roles” above.

After several weeks, the mentor notified the RBL BSO that the trainee had completed 16 training sessions with a total of over 20 hours of training under observation and that the mentor was satisfied with the trainee’s level of proficiency. The RBL BSO and the mentor determined an appropriate procedure for the trainee to perform under observation, a plaque assay. The RBL BSO observed the trainee for one session including facility/locker room entry, suite entry, preparing the biological safety cabinet, performing the plaque assay, decontaminating the liquid and solid wastes and the work surfaces, exiting the suite, and exiting the facility/locker room. The RBL BSO documented the session and then provided the Team with a summary of the trainee’s training plan and accomplishment. The Team unanimously agreed to grant the trainee with independent access to the laboratory and animal suites used by the investigator. The RBL BSO granted the access to the trainee, retained the training checklists in the personnel files, and recorded the date that the trainee received independent access.

Conclusions

The UPitt RBL Management Team undertook a revision of the mentored training program for new investiga-
documentation and communication, and create standardization throughout the program. The work burden has increased for the trainees and particularly for the RBL BSO. This is mitigated by the benefits of the enhanced level of proficiency in lab personnel, improved communication among the Team and mentors, an increased perception of oversight by the Team, consistent documentation of training sessions, and enforceable standards for all new trainees. Following this restructuring, the Team is undertaking a review of the current on-the-job training requirements for veterinary and husbandry technicians as well as for building engineers and is evaluating the best way to align these training programs with the investigative mentored training program.

The responsibility for ensuring that lab personnel are appropriately trained to work at biosafety level 3 is shared by facility managers, biosafety officers, Responsible Officials and investigators. Through assurance that lab personnel are appropriately trained, those who are charged with the oversight of biosafety level 3 research or facilities may gain an additional level of confidence in the reliability and suitability of lab personnel. Therefore, it is beneficial for all of these individuals to participate in the BSL-3 training programs, either by actively participating in bench-top training or by supporting the goals of the training program and those who run it.

Acknowledgment

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References

Biosafety Competencies in Developing Countries: The Role of Universities

Ana Sanchez1*, Jose A. Gabrie1, Ada A. Zelaya2, Lourdes Enriquez2, Maritza Canales2, and Sean G. Kaufman3

1Brock University, St. Catharines, Ontario, Canada, 2National Autonomous University of Honduras, Tegucigalpa, Honduras, and 3Emory University, Atlanta, Georgia

Abstract

With the objective of strengthening biosafety capacities in Honduras, a technical cooperation program has been established with the School of Microbiology, National Autonomous University of Honduras, where most of the country’s infectious disease research and teaching is done. To complete a 2-year cooperation cycle, two significant activities took place in May 2010: the first National Biosafety Meeting in which a Knowledge and Perceptions survey was administered to participants; and secondly, standardized biosafety capacity assessments of several laboratories. Following Emory University Onsite Biosafety Training Program guidelines, assessments evaluated four primary biosafety controls: engineering; personal protective equipment (PPE); standard operating procedures (SOPs); and administrative controls. This technical cooperation program has been successful in revitalizing the school’s biosafety committee and garnering institutional interest. The survey revealed that 57% of respondents did not feel safe in their work environment and that 31% were aware of laboratory-acquired infections in their workplace. Assessments of 12 laboratories showed an overall biosafety grade of 72% and the following specific grades by control: engineering, 73%; PPE, 81%; SOPs, 68%; and administrative controls, 66%. Research laboratories scored consistently higher than their teaching counterparts. Recommendations stemming from these findings have been integrated into the school’s strategic plan. Among other positive changes, the university has allocated a space for a Biosafety Training Center to be launched in the near future. Other efforts towards strengthening biosafety are also underway within the Honduran Ministry of Health. The time is right for Honduras to coordinate efforts leading to the establishment of a nationwide biosafety culture.

Introduction

Biosafety and biosecurity are nowadays considered not only as sets of appropriate practices to be implemented in life sciences laboratories with the aim of protecting personnel and the environment, but also as core values of rigorous, ethical science as well as inextricable components of international research cooperation. Several factors have converged to generate this increasing recognition and the current momentum experienced in the field of biosafety sciences. Speed and volume of travel and commerce, for example, permit the relative ease with which infectious agents can be dispersed (with humans, foodstuffs, or animals) from one side of the planet to the next in a matter of hours (Scotto, 2011). In fact, over the last few years, the world has been preoccupied with the emergence and re-emergence of pathogens and the possibility of pandemics of devastating consequences (IOM & NRC, 2009). This continuing threat has promoted a considerable expansion of transnational scientific and public health networks that permit unprecedented collaboration based on knowledge and technology transfer as well as sharing of pathogens. The latter introduces a new dimension to the globalization of infectious diseases, as these pathogens may find a way out from laboratories. Among other well-known examples, it has been recently hypothesized that laboratory errors may have led to the escape of the 2009 strain of H1N1 influenza A virus from a laboratory with its subsequent re-assortment and spread in the human population (Gibbs et al., 2009).

Similarly, along with current biotechnology advances that permit the production of living modified organisms, the need has arisen to formulate and implement regulations that can be adapted across countries and regions to minimize potential risks that such organisms may pose to ecosystems and human as well as animal health (McLean et al., 2002).

Furthermore, past experiences with the misuse and intentional release of dangerous pathogens have created the need for global-scale efforts leading to the integration of biosafety sciences with biosecurity principles and the advent of the concept of biorisk management (Rudelsheim, 2008). The threat of bioterrorism, however small, carries the potential for immeasurable consequences that transcends public health and endangers global economy and peace. In fact, biosafety and biosecurity have been proposed as essential tools for the stability and development of nations and even entire geopolitical regions (Chua et al., 2009; Nayef et al., 2007; Salerno et al., 2007). All of these factors underscore the urgency of global cooperation and, fortunately, capacity...
strengthening for biosafety and biosecurity are not considered in isolation anymore but as “elements of a greater framework of strengthening global health and security” (IFBA, 2011).

A variety of frameworks and guidelines have been created to assess and strengthen capacities within public health networks and laboratories for they are invested with leadership functions in the event of pandemic containment and bioterrorism readiness (Delany et al., 2011; Marshall et al., 2010). However, even within public health laboratory systems, there exist multiple partners influencing either upstream preparation or downstream decisions and timely actions. Among such partners are the educational institutions that prepare the professionals that would become regulators, leaders, or policymakers. Universities and colleges can and do play a fundamental role in education and training. In this sense, academic laboratories, whether engaged in teaching, research, or both are key stakeholders in promoting a biosafety culture (Lucero et al., 2005). In fact, a systems approach that includes teaching and training is essential to the continual improvement of biorisk management at any level (CEN, 2008).

In developing countries where, despite current efforts, many weaknesses still exist in regard to biosafety and biosecurity practices (Astuto-Gribble et al., 2009), the need for technical cooperation is more pressing than ever.

Honduras is an example of a developing country in which infectious diseases are a leading cause of morbidity and mortality. In addition to the scourge of ancient diseases (many of them among the so-called neglected tropical diseases), the country periodically faces the devastating effects of vector-borne diseases such as Dengue and continues to struggle with one of the highest HIV/AIDS rates in Latin America (with the ensuing increase in tuberculosis cases) (PAHO, 2007; UNAIDS, 2010; United Nations System in Honduras, 2010). Moreover, emerging pathogens such as influenza A H1N1 have made their way into the country underlining the fact that infectious disease outbreaks know no borders and countries must tackle these threats together in order for containment to be effective. As in other countries where the fight against infectious diseases is an ongoing battle, clinical laboratories in Honduras are at the heart of public health activities. Therefore, laboratory personnel at all levels, as well as other health professionals and scientists, must be endowed with the knowledge and skills to work with infectious agents in a safe and secure manner.

In Honduras many of these personnel are trained at the School of Microbiology in the National Autonomous University of Honduras (UNAH), and we have established a technical cooperation program to strengthen research capacity and biosafety practices at UNAH. This article reports and analyzes the outcome and impact of the first external biosafety evaluation undertaken at the School of Microbiology, UNAH, where most of the infectious disease teaching and research in the country are done.

Methodology

Capacity-building Program

In partnership with the School of Microbiology, UNAH, a program dedicated to increasing competencies in biosafety began in 2008. Accordingly, a series of knowledge transfer activities and training sessions have been undertaken. International biosafety experts have visited the country and delivered conferences and skill-based workshops for laboratory-associated personnel from both UNAH and other stakeholders. Additionally, Honduran key personnel have attended international conferences and courses and, in turn, disseminated their knowledge to others upon return. Capacity-building at the School of Microbiology has also included the implementation of a fully equipped biosafety level 2 (BSL-2) research laboratory, the acquisition of biosafety materials and supplies, as well as the facilitation of locally produced printed resources. A key component of the program has been revitalizing the school’s biosafety committee.

To complete a 2-year cooperation cycle, in May 2010 two significant activities took place over a 2-week period: the first National Biosafety Meeting in which a Knowledge and Perceptions survey was undertaken; and secondly, a standardized assessment of laboratory biosafety capacity at the School of Microbiology. These activities are described in detail below.

Knowledge and Perceptions Survey

The First National Biosafety Meeting, a full-day meeting that took place in Tegucigalpa on May 17, was attended by a multidisciplinary audience from several institutions. During this meeting a random sample of participants was surveyed on their knowledge and perception about biosafety. The survey was administered utilizing the audience response system ARS SNAPITM (Audience Response Systems, Inc., Evansville, Indiana, USA). Multiple-choice questions were included within PowerPoint® presentations throughout the day. Participants utilized keypads to report their answers and data were generated simultaneously by ARS SNAPITM software. Questions ranged from descriptions of their workplace, prior training, knowledge of national regulations, risk perception and the workplace, and biosafety-specific knowledge.

Biosafety Capacity Assessment

Biosafety capacity assessment visits were conducted in various BSL-2 research and undergraduate teaching laboratories at the School of Microbiology, UNAH. These assessments followed the terms of reference of the
Emory University Onsite Biosafety Training Program (www.sph.emory.edu/CPHR/biosafetytraining/onsite.html). ONSITE’s data-collecting forms “Assessing Biosafety Programs: Evaluating Laboratory Capacity for Handling Infectious Pathogens” were utilized to conduct the assessments. Forms with detailed information are available upon request.

Four primary controls for biosafety were assessed in each laboratory: engineering; PPE; SOPs; and administrative controls. The assessment of each primary control comprised the evaluation of a defined set of characteristics as depicted in Table 1. The assessment was a two-step process. First, the consultant performed an “external evaluation” while visiting the laboratory, asking a series of standardized questions to the laboratory’s leadership (course instructor, manager, or primary investigator) and/or the staff, as well as by direct observations of the laboratory environment and records. Second, the consultant separately interviewed the lab manager or primary investigator and a member of the staff representing the workforce so they could provide their own assessment or “internal evaluation.” The assessment was done by means of a standardized scoring system using the scale depicted in Table 2. Briefly, percentages or letter grades were assigned to each characteristic within the primary controls. These letter grades were converted to numerical values and added together to average the quality points obtained for each control. Primary control averages would then be added together to generate a final percentage and letter grade for the laboratory. Qualitative interpretations of the final scores can be seen in Table 2. It was agreed that a laboratory would have to achieve an overall 85% score to be designated as having attained a good level of biosafety.

In addition to the laboratory assessments, an inventory of pathogens and biological samples was requested from each lab and an appraisal was conducted of the strengths and improvement opportunities within the School of Microbiology and the university as a whole.

Results

Capacity Building

The overall program has been very successful in revitalizing the biosafety committee and garnering political will from UNAH’s academic administration. Established since 1994, the committee operated at the school level only, with virtually no budget and without an institutional policy to endorse its activities. Its work revolved around establishing good microbiological practices at the teaching laboratories, but since no biosafety training was strictly mandatory for students, staff, or faculty members, the committee had a limited influence over the overall safety practices at the school. Currently, the biosafety committee is highly active and has become a central aspect of the school’s academic life. In particular, cleaning staff and laboratory technicians have re-

Table 1

Defined set of characteristics evaluated for each biosafety control*.

<table>
<thead>
<tr>
<th>Engineering Controls</th>
<th>Personal Protective Equipment (PPE) Controls</th>
<th>Standard Operating Procedures (SOP) Controls</th>
<th>Administrative Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical</td>
<td>Gloves</td>
<td>Evaluation</td>
<td>Training (awareness)</td>
</tr>
<tr>
<td>Security systems</td>
<td>Gowns</td>
<td>Validation</td>
<td>Training (Skills)</td>
</tr>
<tr>
<td>HVAC systems</td>
<td>Lab shoes</td>
<td>Annual review</td>
<td>Medical surveillance</td>
</tr>
<tr>
<td>Alarm systems</td>
<td>Eye protection</td>
<td>Availability</td>
<td>Security clearance</td>
</tr>
<tr>
<td>Physical structure</td>
<td>Respirators</td>
<td>Performance expectations</td>
<td>SOP compliance</td>
</tr>
<tr>
<td>Equipment safety</td>
<td>Ease of PPE</td>
<td>Review process</td>
<td>Staff hiring / screening</td>
</tr>
<tr>
<td>Quality of equipment</td>
<td>Comfort of PPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equipment dependability</td>
<td>Burden of PPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance of equipment</td>
<td>Medical requirements for PPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance of physical structure</td>
<td>Maintenance of PPE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Following the guidelines of Emory University Onsite Biosafety Training Program
Table 2

<table>
<thead>
<tr>
<th>Score (%)</th>
<th>Grade</th>
<th>Quality Points</th>
<th>Qualitative Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-95</td>
<td>A</td>
<td>4.00</td>
<td>Excellent</td>
</tr>
<tr>
<td>90-94</td>
<td>A-</td>
<td>3.66</td>
<td></td>
</tr>
<tr>
<td>87-89</td>
<td>B+</td>
<td>3.33</td>
<td></td>
</tr>
<tr>
<td>86-84</td>
<td>B</td>
<td>3.00</td>
<td>Good</td>
</tr>
<tr>
<td>83-80</td>
<td>B-</td>
<td>2.66</td>
<td></td>
</tr>
<tr>
<td>79-77</td>
<td>C+</td>
<td>2.33</td>
<td></td>
</tr>
<tr>
<td>76-74</td>
<td>C</td>
<td>2.00</td>
<td>Fair</td>
</tr>
<tr>
<td>73-70</td>
<td>C-</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td>69-67</td>
<td>D+</td>
<td>1.33</td>
<td></td>
</tr>
<tr>
<td>66-64</td>
<td>D</td>
<td>1.00</td>
<td>Poor</td>
</tr>
<tr>
<td>63-60</td>
<td>D-</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>59-0</td>
<td>F</td>
<td>0.00</td>
<td>Unacceptable</td>
</tr>
</tbody>
</table>

received significant attention and have participated in various training workshops as well as have been provided with PPE to reduce exposures. A biosafety officer is currently available to students, faculty members, and researchers for consultation on biosafety matters. Importantly, the biosafety officer works together with graduate and undergraduate students engaged in research work to make sure they understand and mitigate the risks associated with their laboratory investigations. Special attention has been given to handling and transporting infectious specimens and other biohazards. In terms of biosafety manuals, although the school has been utilizing the 2004 WHO’s biosafety guidelines (WHO, 2004), it was felt that a locally produced biosafety manual would be also appropriate. Thus, the first Biosafety Manual for the school was published in 2009 and is now widely used among faculty and students.

A major accomplishment of this cooperation program is the implementation of a fully equipped BSL-2 research laboratory named “The Teasdale-Corti Laboratory” in honor of the specific funding program through which the collaboration was established (Figure 1). This entailed extensive renovations of existing spaces and the acquisition of a considerable amount of small and large equipment and supplies (from biohazard bags to autoclaves and other materials, to biosafety cabinets [BSCs] and a fume hood). Also, through small research and teaching grants for faculty members, courses such as quality assurance in the laboratory have been held, teaching laboratories have been renovated, and a significant amount of biosafety materials and supplies has been purchased (including signage, gloves, biohazards disposal materials, etc.). Additionally, an electronic publication frequently showcasing articles on biosafety has been supported (www.revistadelmicrobiolo.org). Experts from the Mexican Association of Biosafety (Amexbio) and Emory University have visited the country and delivered conferences and workshops, and training has been secured for Honduran key personnel in Mexico, Canada, and the United States. More details about these and other capacity-strengthening activities in Honduras can be found at the project’s web site at www.brocku.ca/globalhealth.

Inventory of Pathogens

Teaching and research, the two main activities at the School of Microbiology, entail the determination and/or culture and isolation of human pathogens. A complete inventory of organisms was not available until the biosafety assessment was undertaken. Universally, inventoried pathogens were categorized as Risk Group 2 organisms (WHO, 2004). Among those pathogens, however, cultures of the fungal species Histoplasma capsulatum, Blastomyces dermatitidis, Coccioidioides immitis, and Paracoccidioides brasiliensis, which are classified as Risk Group 3 in other countries (Canada DOJ, 2009), were routinely worked with under open bench conditions.

Knowledge and Perceptions Survey

The First National Meeting was a tremendous success, with 136 people in attendance from various institutions ranging from universities to public health and veterinary laboratories. Eighty-two percent of the participants were women, 38% were either undergraduate or graduate students, 46% were professionals, and 16% were administrators. From this audience, 30 persons were randomly selected to respond to the Knowledge and Perceptions (K&P) survey and to biosafety-specific
questions utilizing the audience response system described above.

Table 3 shows a self-description of the participants (questions 1-3); their familiarity with workplace or national regulations as it pertains to workplace safety biosafety or hazardous waste management (questions 4-6); their perception of occupational risk (questions 7-8); and their opinion on the importance of biosafety training (questions 9-10). It is worth noting that the majority of people who responded that they worked in a BSL-1 laboratory actually worked in a BSL-2 facility. In regard to training, almost half of the respondents did not attend biosafety instruction in the past year, but all expressed interest in receiving different formats of training. More than half of the participants (57%) felt they were not protected in their workplace and 31% were aware of laboratory-acquired infections in their workplace. The corresponding question to the latter finding implied the respondent’s lifetime experience and did not require further details from the participant.

Table 4 shows biosafety-specific knowledge of the polled individuals. As questions were posed in the context of a talk related to the topic, the level of knowledge was considerably high. Very importantly, people felt a strong connection between science and safety and were fairly conscious that human behavior is the key to the safe handling of biological hazards.

Biosafety Capacity Assessment of Teaching and Research Laboratories

Laboratory assessments were undertaken in 12 out of 17 existing laboratories. Of the 12 assessed, five were mainly dedicated to research and seven to teaching. One laboratory (labeled number 2 on the figures) did not have permanent staff to be interviewed, so the average was adjusted accordingly.

Interviews took place after several activities for biosafety strengthening had taken place. In particular, the consultant facilitated some workshops and gave several talks on BSL-2 practices and also on the role of human behavior in laboratory safety. This allowed for a better understanding by the leadership and staff of the concepts and terminology used for the assessments. Moreover, since the interviews to obtain the internal assessments by the leadership and staff were face-to-face with the consultant, there was ample opportunity for further clarification.

The average grade for all 12 laboratories was 72% (SD = 5%). For each biosafety control, the average results were: engineering, 73%; PPE, 81%; SOPs, 68%; and administrative controls, 66%. Figure 2 shows the average percentages for each laboratory by biosafety control. Laboratories numbered 1-5 were dedicated to research whereas laboratories 6-12 to teaching. Considering the thresholds explained above, laboratories were universally below a good level of biosafety (<85%) but above the unacceptable level (≤59%).

Figure 3 displays assessments for laboratories showing the scores estimated for leadership, staff, and the consultant. Notwithstanding the training occurring around the interviews, there was a difference in the external assessment (i.e., consultant) as compared to the internal one (leadership and staff).

Research Versus Teaching Laboratories

When categorized as a research or teaching laboratory, interesting differences were revealed. Research laboratories scored consistently higher in every respect.
Table 3
Self-description of Participants: Knowledge on regulations and perceptions about occupational risk and biosafety training among participants to the First National Biosafety Meeting, Honduras, May 17, 2010 (n = 30).

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer Options and % of Responses</th>
<th>Comment/Correct Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. In what biosafety containment level (BSL) do you work?</td>
<td>BSL-1: 30%</td>
<td>The majority of participants actually worked for clinical laboratories where biological specimens and laboratory cultures are handled, so they would technically work in BSL-2.</td>
</tr>
<tr>
<td></td>
<td>BSL-2: 26%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BSL-3: 0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable: 30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I don’t know: 15%</td>
<td></td>
</tr>
<tr>
<td>2. In how many biosafety talks, trainings, or courses have you participated in the last year?</td>
<td>0: 43%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1: 39%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2: 4%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3: 9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4: 4%</td>
<td></td>
</tr>
<tr>
<td>3. In what type of biosafety education/training would you be interested in participating annually?</td>
<td>One or two short talks or seminars: 30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A short course &lt;20 hours: 30%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>An intensive course 1-2 weeks: 20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 100-hour diploma course: 20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I don’t know: 0%</td>
<td></td>
</tr>
<tr>
<td>4. How well do you know the occupational health and safety policy of your workplace?</td>
<td>Very well: 4%</td>
<td>Most workplaces in Honduras do have occupational health policies as the law mandates them to have them in place.</td>
</tr>
<tr>
<td></td>
<td>Not well but there is one: 54%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I know there is a policy but I have never read it: 38%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I believe there is no such policy in my workplace: 4%</td>
<td></td>
</tr>
<tr>
<td>5. Are you aware of the existence of biosafety guidelines or regulations in your workplace?</td>
<td>Yes: 72%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No: 28%</td>
<td></td>
</tr>
<tr>
<td>6. Are you aware if there is a law in Honduras to regulate hazardous biomedical and infectious waste?</td>
<td>Yes: 37%</td>
<td>In July 2008, the Honduran government through the Ministry of Health approved legislation for the appropriate handling and disposal of hazardous waste. It became effective on February 28, 2009 (Honduras Jurisprudence and Legislation, 2008).</td>
</tr>
<tr>
<td></td>
<td>No: 63%</td>
<td></td>
</tr>
<tr>
<td>7. How protected do you feel from acquiring an infection while performing your job?</td>
<td>Protected: 37%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unprotected: 56%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I don’t know: 7%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable: 0%</td>
<td></td>
</tr>
<tr>
<td>8. Are you aware of any laboratory-acquired infection in your workplace?</td>
<td>Yes: 31%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No: 69%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable: 0%</td>
<td></td>
</tr>
<tr>
<td>9. In your opinion, which of the following is the single most important element of a biosafety program?</td>
<td>Personal protective equipment: 12%</td>
<td>Most experts would agree that behavior and training are the single most important elements (Kaufman et al., 2007).</td>
</tr>
<tr>
<td></td>
<td>Training: 60%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Equipment and physical facilities: 8%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Regulations: 20%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I don’t know: 0%</td>
<td></td>
</tr>
<tr>
<td>10. In your opinion, how important is biosafety training as part of the overall best practices and quality assurance of your laboratory or workplace?</td>
<td>Very important: 97%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderately important: 3%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not too important: 0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>It doesn’t make any difference: 0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I don’t know: 0%</td>
<td></td>
</tr>
</tbody>
</table>

BSL: biosafety level
### Table 4
Biosafety-specific knowledge displayed by participants at the First National Biosafety Meeting, Honduras, May 17, 2010 (n = 30).

<table>
<thead>
<tr>
<th>Question</th>
<th>Responses in % (Correct response shown in bold)</th>
</tr>
</thead>
</table>
| 1. How strong do you believe is the relationship between science and safety? | Very strong: 80%  
Moderate: 20%  
Not much: 0%  
No relation: 0%  
I don’t know: 0% |
| 2. Having a biosafety manual guarantees good laboratory practices.      | Yes: 32%  
No: 68% |
| 3. How important is having a medical surveillance program for minimizing risks? | Very important: 100%  
Moderately important: 0%  
Not too important: 0%  
It doesn’t make any difference: 0%  
I don’t know: 0% |
| 4. Who is responsible for the adherence to biosafety regulations in the laboratory? | Administrators: 0%  
Biosafety Officer: 12%  
Lab Director: 4%  
Lab Technicians: 0%  
Everybody: 84% |
| 5. Besides knowledge and resources, what is another factor necessary to ensure sustainable behavior? | Understanding risks and benefits of change: 88%  
Regulations and laws: 12%  
I don’t know: 0% |
| 6. How much weight does human behavior bear on expected results?        | Considerable weight: 100%  
Moderate: 0%  
Not much: 0%  
None: 0%  
I don’t know: 0% |
| 7. The following is an example of personal protective equipment:         | Biosafety cabinet: 11%  
Gloves: 82%  
Decontamination procedures: 7%  
Standard operating procedures validation: 0% |
| 8. The following is an example of biosafety administrative control:       | Biosafety cabinet: 4%  
Gloves: 4%  
Decontamination procedures: 19%  
Standard operating procedures validation: 73% |
| 9. True or False: All work in a BSL-2 laboratory must be done inside a biosafety cabinet. | True: 26%  
False: 74% |
| 10. A sophisticated facility with strong engineering controls ensures protection in the daily laboratory work. | Yes: 0%  
No: 100% |

compared to their teaching counterparts, for an overall grade of 77% versus 68%, respectively. When investigating the differences, it was found that results were fairly similar in the administrative controls (68% vs. 65%) but differed remarkably in the remaining three controls (Figure 4).

All research laboratories were collaborating with international partners, which provided funding for renovations and upgrades, purchase of new equipment (including BSCs), and sufficient supply of PPE. As well, due to the nature of the investigations carried out, research laboratories had a good collection of SOPs and laboratory techniques. The teaching laboratories, on the other hand, relied entirely on the institution’s resources. For example, none of the teaching laboratories had BSCs, some lacked appropriate storage spaces for pathogen collections, and all struggled with securing a steady supply of PPE and appropriate hazardous waste disposal materials.

**Overall Assessment by Biosafety Control**

**Engineering Controls:** As seen in Table 1, the engineering assessment entailed appraisal of structural aspects beyond the power of individual laboratories (such as electricity and facilities management) as well as laboratory equipment and maintenance. Frequent power
Figure 2
Biosafety scores by each of the four controls for 12 university laboratories in Honduras.

*Research labs: 1-5; teaching labs: 6-12

Figure 3
Comparison of average biosafety scores assigned by staff, leadership, and consultant for 12 university laboratories in Honduras.

*Research labs: 1-5; teaching labs: 6-12; †There is no permanent staff in lab 2
outages occur in the city, and with the lack of emergency power generators at the institution, laboratories are left without power on a regular basis. Also, facilities maintenance was reported to be slow and unreliable and there were persistent problems such as roof leakage and general physical upkeep. Although these situations were similar for both research and teaching laboratories, due to external funding availability, the former were able to have better working conditions (although several principal investigators reported resorting to personal funding to install locks, paint, or have equipment repaired). Most teaching laboratories lacked air conditioning, which forced staff to work with open windows (often without screens).

**PPE Controls:** Unavailability of disposable gloves was a prominent problem for the teaching laboratories and students were required to supply their own gloves during laboratory practices. It was noted that disposable facemasks were often cited as being used by lab technicians and students when working with clinical samples and bacterial cultures for splash protection as well as to block odors. N95 disposable respirators were used in some research laboratories, although the work being done was not deemed to require respiratory protection. It is also worth mentioning that laboratory coats were consistently worn outside the BSL-2 laboratories. This was especially true for students, who were observed wearing their laboratory coats everywhere, including off campus. Also, as no laundry facilities are available at the university, all personnel reported bringing their lab coats home to launder. Additionally, although wearing open-toe shoes in the BSL-2 labs was discouraged, not everybody complied with this safety practice.

**SOP Controls:** Research laboratories had binders with lab techniques and teaching laboratories had manuals to conduct practical sessions. However, neither research nor teaching laboratories had available SOPs nor manuals for good microbiological techniques, including house rules (laboratory code of conduct), safe handling of specimens, spill containment procedures, etc. No SOPs were available for autoclaving, cleaning contaminated material, transporting biohazards from teaching laboratories to the autoclave room, disposing of biological specimens, etc. The lack of appropriate spaces for personnel to store and consume their food and to wash their utensils was revealed to be a serious problem during the onsite assessment. Food was sometimes stored in laboratory refrigerators and utensils washed in laboratory sinks (Figure 5).

**Administrative Controls:** As mentioned, at the time of the interviews no institutional biosafety guidelines and regulations existed at UNAH, and despite noble efforts from the biosafety committee members, there was no firm strategy to ensure full compliance with international guidelines. Most BSL-2 laboratories had a biohazard sign on the door, but the type of hazard and protection required were not specified. No laboratory kept records of incidents or accidents and many of the interviewees were not aware of the occurrence of laboratory-acquired infections. Similarly, medical surveillance protocols were
unheard of among interviewees, and, in some cases, their implementation was deemed unnecessary, as most pathogens with which they worked are endemic to the country. Emergency/contingency plans were non-existent at the local or institutional level. Importantly, cognitive training (e.g., seminars and talks) on biosafety was held with some regularity and most courses with a laboratory component had integrated one biosafety awareness session into their curriculum.

Based on all these findings, on October 2010, a full report was provided to the School of Microbiology and other senior administrators, including the Dean of Faculty of Sciences. The report contained a set of 20 recommendations as to how to strengthen biosafety capacities at the school so it could operate more safely, and in time, lead university-wide efforts. Additionally, each participating laboratory was provided with an individual report containing specific recommendations. Recognizing that attaining a sustainable higher level of biosafety requires collective ownership from all stakeholders, the biosafety committee conducted a detailed analysis of the report with staff and faculty members so together they could propose and be responsible for the achievement of common goals.

**Institutional Response to the Assessment**

Following the biosafety assessment and recommendations provided in 2010, the School of Microbiology has developed a strategic plan 2011-2014 and has taken immediate actions such as: strengthening the school’s biosafety committee; developing standard operating procedures SOPs; improving waste management (with the aid of the Swedish Cooperation); and holding students’ training and awareness events regularly. A follow-up assessment visit is planned for 2012. Additionally, the Faculty of Sciences has allocated physical space for a Biosafety Training Center, which will be managed by the biosafety committee. At the moment, the biosafety committee is drafting a work plan to launch the Center with a “train the trainers” component. At the same time it has recruited international experts to assist with developing a portfolio of courses and training opportunities for a wide array of professionals and students.

**Discussion**

The results of the survey—although lacking scientific validity—revealed important gaps in knowledge and training among the audience. Of special concern was that 31% of the participants reported knowing of a laboratory-acquired infection in their workplace. As mentioned, the corresponding question to this finding implied the respondent’s lifetime experience and did not require the participant to elaborate on his or her answer. Since clinical, teaching, or research laboratories in Honduras do not keep track of accidental exposure to pathogens, there is no available data to corroborate this finding. Therefore, it must be interpreted with caution. However, this finding alone merits an in-depth investigation and should prompt employers and laboratory managers to implement annual biosafety trainings as well as incident reporting and medical surveillance programs. Another important finding was that, unknown to the majority of participants, since 2009 Honduras has had national regulations for the safe handling and disposal of hazardous waste (infectious, chemical, and radioactive) produced at medical facilities, clinical and forensic laboratories, blood banks, universities, funerary services, cemeteries, and others. According to the regulations, if hazardous waste cannot be rendered innocuous at the pri
municipal land or public cemeteries. Naturally, compliance with these regulations will require certain conditions to be met such as having the knowledge and capacity (administrative, material, and human resources) at the primary facilities where waste is produced. These conditions are yet to be developed in most facilities in Honduras.

The findings from the capacity assessment of teaching and research laboratories at the School of Microbiology showed a fair degree of biosafety awareness among the interviewees and revealed excellent opportunities for improvement. For example, some laboratories were doing relatively well in terms of PPE and equipment controls as opposed to SOPs and administrative controls. Since writing and validating SOPs and implementing biosafety training and SOP compliance require less economic investment and more commitment and institutional support, leaders from participating laboratories were amenable to making short-term changes to increase biosafety in their laboratories and, by the same token, the security of the pathogens in the laboratories. One of the main recommendations stemming from the assessments was the articulation of contingency plans and emergency response strategies, with special attention to formulating SOPs for working in the laboratory under power outages, equipment breakdown, and PPE scarcity. Further, as the assessments performed with the Onsite program did not account for risk, laboratory leaders were also encouraged to conduct risk assessments to determine appropriate laboratory-specific levels of control and the ensuing risk management strategies.

The assessments also revealed striking differences between research and teaching laboratories. On one hand it was encouraging to see that research laboratories operating fairly independently from the institution, were well equipped and did not suffer from a serious lack of PPE. On the other hand, it was disconcerting to observe the little attention given to teaching laboratories. Besides teaching their own students, the School of Microbiology is tasked with teaching microbiology and parasitology to a wide array of undergraduate students including medicine, nursing, dentistry, and pharmacy students. Therefore, another important recommendation made to the school was to implement a university-wide biosafety training program mandatory for all those students as well as other training opportunities for a variety of stakeholders both inside and outside the university. As expressed by a participant in one training session, “There are many opportunities for instilling basic biosafety principles in a variety of students, professionals, and administrators, and the rewards for doing so are endless. If we work together, we can all be safer.” It is well known that in Cuba, individual researchers first, and a research institute later, were essential in spearheading a national biosafety movement (Fernández et al., 2002). High hopes exist that in Honduras this important role belongs to UNAH.

Similarly, there is optimism that scientific research in the country is becoming a point of interest among policymakers and government officials. Through its Health Minister representing the country at the Council of Ministers of Health of Central America (COMISCA), Honduras has recently recognized the role of research and science as a tool for development and, along with other countries in Central America and the Caribbean, is committed to the “establishment and strengthening of national systems for health research, so as to increase the production and utilization of research activities that address health needs, equity, and development of the countries of the Subregion” (COMISCA, 2009). In light of this commitment, it is anticipated that a concerted effort will hopefully entail strengthening of the several pillars of health research, including biosafety.

Opportune to this progress, the present collaboration (part of a 5-year project aiming to strengthen Honduras’ capacity to do research on and respond to infectious and zoonotic diseases) has brought to UNAH, the largest academic institution in the country, the opportunity to advance in the area of biosafety so it can, by developing the appropriate expertise, fulfill its mandate to be directly involved in biosafety and biosecurity issues. Mandate and expertise are two of the three characteristics for a successful multi-stakeholder partnership identified in the recent declaration by the International Federation of Biosafety Associations (IFBA) for the global advancement of biosafety and biosecurity (IFBA, 2011). With the experience gained, UNAH is now ready to leverage national and international cooperation to help it crystallize its nascent culture of biosafety. Additionally, UNAH has the potential to become a key player in the area of biosafety in the country since considerable efforts are already taking place at the central level. For instance, with major support from the Pan American Health Organization, the National Laboratory Network has upgraded biosafety practices at various virology laboratories and published in 2010 its first Biosafety Manual for Clinical Laboratories. Harmonization of efforts between UNAH and the Ministry of Health with active collaboration of professional associations such as the colleges of microbiologists and laboratory technologists would yield significant results that may certainly have national and Central American impact. Such partnership has already proven extremely successful in Argentina, where the implementation of several coordinated strategies since the early 1990s has resulted in a country-wide enhancement of biosafety practices worth replicating in other developing countries (Lucero et al., 2005).

At the national level, Honduras would benefit greatly from implementing a systematic and participatory process leading to the establishment of national biosafety regulations. Arguably, regulations per se will not guaran-
tee compliance. Indeed, the cornerstone for the safe practice of science is human behavior. But regulations and compliance-monitoring, complemented with several training modalities including competency-based (Delany et al., 2011) and behavioral-based (Kaufman et al., 2007) as well as continued awareness campaigns, would elicit a synergistic effect that will facilitate the work of biosafety committees and biosafety officers whose undertaking will be far more comprehensive than anyone can anticipate (Kaufman et al., 2007).

Continued leadership and national and international support over a period of more than 25 years has converted Cuba into one of the finest models for biosafety in Latin America. During almost the same period, national legislation and multi-partner coordination has contributed to Argentina’s exemplary culture of biosafety. With the current globalization trends, however, neither the developing countries weak on biosafety nor the countries that might be affected by such weakness can afford to wait a quarter of a century for acceptable levels to be reached. The time has come for Honduras to take action and strengthen biosafety and biosecurity practices for its own and the global good. In the complex network of numerous partners, stakeholder UNAH, with its new Biosafety Training Center, seems to be well suited to play an agglutinating function and a fundamental role in capacity building.

Acknowledgments

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References


Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories: Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel

Supplements
www.cdc.gov/mmwr/pdf/other/su6101.pdf

Summary
Prevention of injuries and occupational infections in U.S. laboratories has been a concern for many years. CDC and the National Institutes of Health addressed the topic in their publication Biosafety in Microbiological and Biomedical Laboratories, now in its 5th edition (BMBL-5). BMBL-5, however, was not designed to address the day-to-day operations of diagnostic laboratories in human and animal medicine. In 2008, CDC convened a Blue Ribbon Panel of laboratory representatives from a variety of agencies, laboratory organizations, and facilities to review laboratory biosafety in diagnostic laboratories. The members of this panel recommended that biosafety guidelines be developed to address the unique operational needs of the diagnostic laboratory community and that they be science based and made available broadly. These guidelines promote a culture of safety and include recommendations that supplement BMBL-5 by addressing the unique needs of the diagnostic laboratory. They are not requirements but recommendations that represent current science and sound judgment that can foster a safe working environment for all laboratory personnel.

Throughout these guidelines, quality laboratory science is reinforced by a common-sense approach to biosafety in day-to-day activities. Because many of the same diagnostic techniques are used in human and animal diagnostic laboratories, the text is presented with this in mind. All functions of the human and animal diagnostic laboratory—microbiology, chemistry, hematology, and pathology with autopsy and necropsy guidance—are addressed. A specific section for veterinary diagnostic laboratories addresses the veterinary issues not shared by other human laboratory departments. Recommendations for all laboratories include use of Class II A2 biological safety cabinets that are inspected annually; frequent hand washing; use of appropriate disinfectants, including 1:10 dilutions of household bleach; dependence on risk assessments for many activities; development of written safety protocols that address the risks of chemicals in the laboratory; the need for negative airflow into the laboratory; areas of the laboratory in which use of gloves is optional or is recommended; and the national need for a central site for surveillance and nonpunitive reporting of laboratory incidents/exposures, injuries, and infections.
Ask the Experts
John H. Keene
Global Biohazard Technologies, Inc., Midlothian, Virginia

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

Some Researchers Claim That Their Laboratories Must Be Under Positive Pressure to Keep Unwanted Materials from Entering and Contaminating Their Work. Is It Appropriate to Have Positively Pressurized Laboratories for Any Reason?

While it is sometimes difficult to insure, all laboratories should be negative to the corridors. The reasons for making the laboratory negative to the corridor are many and compelling. Laboratorians use hazardous volatile chemicals that need to be prevented from exiting the laboratory into the corridor. Animal dander and other allergens need to be contained in the animal rooms and removed by ventilation. Biological aerosols, probably the largest of the hazards if we are working in biosafety cabinets (BSCs), need to be removed from the laboratory should a spill occur outside of the primary containment device.

Let’s face it, we are doing research in these laboratories and some level of hazard is always present. That is the nature of the research business. Researchers will always be at some risk of exposure to those hazards, but people in the corridors should not be at high risk. Therefore, directional airflow from corridors to labs and out of the building is appropriate. In addition, negative pressure in laboratories helps to contain those infrequent but ever-persistent laboratory fires that may occur.

The tissue culture people, and now the Polymerase Chain Reaction (PCR) people, are continually worrying about protecting their work and rightly so, but there are ways to prevent potential contamination. The time to think about that possibility is not after the contamination happens, but before it happens. You can protect work in a number of ways (think outside the box a little: biosafety cabinets, ventilated animal cages, pressurized air locks, better technique, etc.).

Proper use of biosafety cabinets, which, by the way, were designed to, and in fact do, protect not only the worker but also the work, can go a long way towards insuring that the work is properly protected. My experience is that many laboratorians complaining about outside contamination of their work are not properly trained in the use of the BSC. This includes many of the principal investigators who have been in the laboratory for years. Failure to use proper technique during operations within a BSC is, in our experience, the most common cause of contamination of tissue cultures and other work performed in the BSC. The cabinets themselves rarely are the problem. In addition, potential contamination in the laboratory itself is also a rare factor in contamination of such cultures.

Animal dander allergy has become a significant problem in the research arena. Ventilated and filtered animal cages can be used to protect immunosuppressed animals while also protecting personnel from animal dander allergies. When using such cages to protect the environment from contamination with agents from infected animals, it is important to have the cage manufacturer provide information on how the filters are tested to insure integrity and guidance on how to decontaminate the units prior to servicing.

Finally, new laboratories can be designed with air locks that are at positive pressure to both the corridor and the laboratory. Such air locks can be supplied with HEPA-filtered air to minimize contamination of the laboratory from outside, which may be appropriate for PCR and other genetic engineering work but is generally not necessary otherwise. Unfiltered positive-pressure air locks are frequently used in areas where the facility opens directly to the outdoor environment and the high humidity in the outside air must be kept from entering directly to an air-conditioned space.
Animal Bytes
Barbara Johnson
Biosafety Biosecurity International, Herndon, Virginia

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 Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Small Animal Containment Caging

Conducting work at ABSL-3 has the potential to increase the risk of exposure to pathogens in animal care-takers, laboratory workers, maintenance personnel and other individuals handling animals, cages, waste, or working in the room. Preventing exposure to pathogens at the source is a basic tenet in biosafety with examples including primary containment devices such as biosafety cabinets, sealed centrifuge cups and individually ventilated cages (IVC). This column provides an overview of several commercially available murine containment caging options that may be used while working in containment.

Disposable HEPA Filtered Cages

Disposable cages are used once and then autoclaved and either incinerated or recycled. When working with prions, best practices recommend that cages are incinerated after use. Like conventional cages, disposable cages with HEPA filter barriers can be used on open racks or as part of an IVC rack system. They are usually constructed of polyethylene or polystyrene, and are irradiated and bagged to ensure they remain sterile. They are attractive alternatives to traditional caging from a couple of unique perspectives including (1) reduced labor and (2) simplification of operations (Fallon, 2011).

When using disposable cages in ABSL-3, the operational flow encompasses:
- Deliver to loading dock → store as sterile cages → use in ABSL-3 animal holding room → autoclave → dispose or recycle.

When working with reusable cages, the operational flow can have additional steps that include:
- Store as clean cages → autoclave → use in ABSL-3 animal holding room → autoclave → use dumping station/cage wash → refill with bedding → clean storage.

Depending on the degree of automation, the dumping station/cage wash operation may involve manual dumping, cage scraping, and loading/unloading the cage wash or the use of an automated dump/wash robotic system that reduces many of the manual steps. Whether using disposable cages actually reduces exposure hazards to animal care personnel depends on a number of factors and can be determined only by conducting a risk assessment. For example, the likelihood of exposure to infectious agents due to cage type and handling should be equivalent in a situation where air from each type of cage (disposable vs. reusable) is HEPA filtered, the cages are placed on a ventilated rack, the cages use a similar watering system, the lids are securely sealed to the cage during transport, and the cages exit the ABSL-3 via the autoclave. If one of these assumptions is invalid, then there is a potential risk for exposure to pathogens. For example, if one type of cage has a lid that cannot be securely fastened to the cage, personnel may be exposed due to the lack of a lid seal or a displacement of the lid when the cage is bumped or dropped. Securely sealed means fail-safe leak tightness under normal ABSL-3 operations and accidents such as dropping. This is important for any type of cage system in an ABSL-3. In addition, risk of exposure can be increased by poor procedures. Regardless of whether cages are disposable or reusable, it is vital that the autoclave cycle be validated for the cage and bedding type, number of cages being autoclaved at one time, and how they are stacked inside the autoclave prior to dumping bedding or disposing of potentially infectious materials.

The economic advantage of using either disposable or reusable cages varies based on the institution. Costs depend in part on the number of cages needed over a given period of time, cost per cage type, capital equipment and utilities, and labor. In addition, purchasing irradiated cages containing bedding can further reduce costs associated with labor and the conditioned storage space needed for consumables. As increasing importance is placed on “green” facilities, the question of environmental impact associated with each type of cage should be considered. Factors include: the energy and resources required to manufacture, dispose of, or recycle the cage; local availability of recyclers; and water and energy requirements for cleaning the cages.

General Features of Cages and Racks

Several cage and rack features are important to provide primary containment. Lids of free-standing cages should be equipped with HEPA filters to prevent the release of pathogens into room air. A continuous gasket between the lid and the body of the cage is recommended to provide a seal that prevents air exchange. In the case of reusable cages, the gasket should be sufficiently
durable and made of materials that withstand autoclave temperatures and chemicals used in the cage-washing process. There should be a means of securely fastening the lid to the cage, and in the case of cages housed on racks, securing the cage to the rack. Cages used in rack systems may be individually HEPA-filtered at the cage level as well as at the rack level, providing a hermetic environment. Those that are not individually HEPA-filtered should be equipped with sealed air supply and exhaust ports to prevent release of contaminants when cages are removed from the rack. Cages may have pre-filter on the exhaust to prolong the life of the rack system HEPA filters.

IVC racks have to be sturdy and able to withstand the relatively rough handling associated with cage changing, cleaning and movement in the room or through the facility. Consideration should be given to cleaning and sanitizing racks on a regular basis. If racks cannot be autoclaved out of containment for maintenance, repair, or decommissioning, an airlock for gaseous decontamination of the racks should be available in an ABSL-3. Because IVC racks have HEPA-filtered supply and exhaust, they should be certified annually just as the biosafety cabinet is certified. The rack ventilation system should be negative relative to each cage (providing protection between cages) and the room (providing environmental protection). As the rack is a system that delivers filtered air to and from the cage, it is important that the connections between the HEPA filters and the air delivery system remain sealed. An alarm system with a battery back-up is a must for IVC rack systems as animals are dependent on the functioning rack for their air supply. The alarm system should be able to contact animal facility personnel immediately in the event of a power or equipment failure. The battery back-up or uninterrupted power supply provided by the facility should be able to provide airflow until personnel responding to the alarm can identify and correct the problem (Sidelsky, 2007). Since emergencies may occur during off-hours, such as holidays, weekends, and evenings, thought should be given to the time involved in commuting to the facility and accessing the containment area when deciding on battery life.

Recirculating and Hard-ducted Racks
IVC racks may be free-standing and recirculate HEPA-filtered air into the room or hard-ducted to the building HVAC. In both instances the movement of air across the bedding helps to dry the bedding and remove waste gases, reducing the frequency of cage changing and handling. Advantages to free-standing racks include the flexibility to use them in different rooms and configurations and their ease of movement throughout the facility. While it seems intuitive that recirculating air back into the room would yield high energy savings, this is not the case because the recirculated air contains ammonia and carbon dioxide from the cage. This may prevent substantial reductions in the number of room air changes per hour (AC/H).

Advantages to hard-ducting racks include the enhanced removal of odor from the room and the potential for added personnel protection if the unit exhaust HEPA filter is breached. Additionally, energy saving advantages of hard-ducted IVC racks were described by Brown and Trent as part of Harvard University Campus Greening Initiative to evaluate and implement vivarium energy saving measures (Brown et al., 2008). While the Institute for Laboratory Animal Research (ILAR, 2008) recommends the provision of 10-15 AC/H to maintain air quality in the room, it also notes there are many variables to consider in determining the correct set point including but not limited to the use of IVC racks. One of the conservation opportunities the Harvard Initiative report focused on involved lowering the building ventilation rates in animal holding and procedure rooms (from 12-15 AC/H to 8-10 AC/H) and in IVC (from 60 AC/H to either 50 AC/H or 42 AC/H) (Brown et al., 2008). The test bed consisted of two rooms that were representative of animal holding and animal breeding rooms. In addition to measuring energy consumption, differences in soiled cage changing interval, breeding efficiency and animal behavior, and changes in cage and room temperature and relative humidity were also studied. Brown and Trent (2008) reported no measurable changes in any of the parameters studied except for an increase in energy savings. The results showed an estimated ventilation reduction of 3,000 CFM, yielding an estimated savings of $24,000 per year. This portion of the project cost just under $12,000 and was “paid back” within 6 months of operation.

It is important to ensure that any changes made to the facility ventilation system do not adversely affect the animals or people working in the rooms and that cages are appropriately selected for the hazards associated with the project. The Institutional Animal Care and Use Committee and Office of Health and Safety are a valuable resource for guidance on optimizing cage and rack systems.

References


Capsule

Felix K. Gmuender
Basler & Hofmann Singapore Pte Ltd., Singapore

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.

Update on Vaccine-derived Polioviruses, Evaluation of RT-PCR for Differentiation of Mycobacteria tuberculosis Complex, Transduction of Human Cells with Polymer-complexed Ecotropic Lentivirus, and Transmission Dynamics of Pneumonic Plague

Update on Vaccine-derived Polioviruses—Worldwide, July 2009-March 2011

CDC (2011) reports that in 1988, the World Health Assembly resolved to eradicate poliomyelitis worldwide. The live, attenuated oral poliovirus vaccine (OPV) has many advantages favoring its use in polio eradication: It is administered easily by mouth; confers intestinal immunity, making recent OPV recipients resistant to infection by wild polioviruses (WPVs); provides long-term protection against paralytic disease through durable humoral immunity; and is inexpensive. Despite its many advantages, OPV use carries the risk for the rare occurrence of vaccine-associated paralytic poliomyelitis among immunologically normal OPV recipients and their contacts and the additional risk for emergence of vaccine-derived polioviruses (VDPVs). Because of these risks, OPV use will be discontinued worldwide once the goal of eradicating all WPV transmission is achieved. VDPVs can cause polio outbreaks in areas with low OPV coverage and can replicate for years in immunodeficient persons; therefore, strategies to strengthen global polio immunization and surveillance are needed to limit the emergence of VDPVs. This report updates previous surveillance summaries and describes VDPVs detected worldwide from July 2009 to March 2011 and reported as of June 20, 2011. Three new outbreaks of circulating VDPVs (cVDPVs), ranging in size from 6 to 16 cases, were identified in Afghanistan, Ethiopia, and India; three previously identified outbreaks in Nigeria, Democratic Republic of Congo (DRC), and Somalia continued through late 2010 or into 2011 and resulted in 355, 37, and 13 total cases, respectively; two countries experienced importations of cVDPVs from Nigeria; nine newly identified paralyzed immunodeficient persons in seven middle-income and developing countries were found to excrete VDPVs; and VDPVs were found among persons and environmental samples in 15 countries. With the use of alternate OPV formulations since 2005 and with enhanced poliovirus surveillance sensitivity and laboratory screening, the number of identified cVDPV outbreaks per year has increased over time. To prevent VDPV emergence and spread, all countries should maintain high poliovirus vaccination coverage against all three poliovirus serotypes. Sensitive poliovirus surveillance to detect VDPVs will continue to increase in importance.


Evaluation of a Single-tube Multiplex Real-time PCR for Differentiation of Members of the Mycobacterium tuberculosis Complex in Clinical Specimens

The differentiation of Mycobacteria species within the Mycobacterium tuberculosis complex (MTBC) is important for public health surveillance and reference testing. The Mycobacterium tuberculosis complex includes the closely related M. tuberculosis, M. africanum, M. bovis, M. bovis BCG, M. microti, M. canetti, M. caprae, M. pinipedi, and M. mungi. Most of these species are able to infect humans. M. bovis has a reservoir in animals. Halse et al. (2011) report that the existing methods to rapidly differentiate MTBC are not always suitable, limited in terms of species range or not validated for use on clinical specimens. No assay is available to perform MTBC differentiation directly from clinical specimens. The authors have developed a real-time polymerase chain reaction (RT-PCR) assay that meets these requirements. The test is built on the presence or absence of regions of difference (RD) between the genomes of members of the MTBC. This allowed the research team to design a single tube, fiveplex, real-time Polymerase Chain Reaction (PCR) to identify six of the MTBC species, namely M. tuberculosis, M. bovis, M. bovis BCG, M. africanum, M. microti, and M. canetti. The performance of
the assay was evaluated by testing 192 MTBC-positive clinical specimens both with the new assay and with conventional cultural methods. A 94% correlation was found. Additionally, a 97% correlation was noted when the new assay was compared with 727 Bactec MGIT 960-positive cultures. Halse et al. (2011) conclude that the new test can be used directly on clinical samples, is inexpensive, produces results within 2.5 hours, is performed in a closed-format system, and requires minimal manual handling.


Transduction of Human Cells with Polymer-complexed Ecotropic Lentivirus for Enhanced Biosafety

Pantropic viruses are often used for stem- and tumor-cell studies that require viral transduction of human cells with known or suspected oncogenes. Pantropic viruses, such as the often used VSV-G pseudotype, are capable of infecting various hosts (mammals, insects, amphibians). Protocols involving panotropic viruses to encode oncogenes typically require a higher biosafety level, such as BSL-2+ or BSL-3, according to the pertaining institutional policies and biosafety regulations. In contrast, ecotropic viruses may readily infect only mouse or rat cells. Barrilleaux and Knoepfler (2011) demonstrate the use of an ecotropic lentivirus for the overexpression of oncogenes in human cells, thereby reducing biosafety risks and at the same time increasing the transduction rate. Human target cells become susceptible to the ecotropic lentivirus only after transduction with a human lentivirus that encodes for the murine (ectotropic) retrovirus receptor. This protocol obviates the need for ultracentrifugation, a time-consuming and, in this context, potentially unsafe procedure. As an alternative to ultracentrifugation, the authors present a sedimentation step to concentrate the viral particles. The sedimentation rate is remarkably accelerated by complexing the virus with the polymers chondroitin sulfate and polybrene. The online paper includes a video demonstration of the protocol.


Transmission Dynamics of Primary Pneumonic Plague in the USA

The bacterium Yersina pestis, causing the life-threatening disease plague, is thought to be a prime candidate for potential use as a bioweapon. The disease manifests in three clinical forms: bubonic, septicemic, and pneumonic. The pneumonic form, which is considered the most serious form, is caused by the inhalation of bacteria, such as when an unprotected person is exposed to a coughing or sneezing patient, or when aerosolized bacteria are inhaled. Pneumonic plague is the only clinical form that is transmissible from person to person. Y. pestis is circulating in rodents in many parts of the world and can be retrieved, isolated, and multiplied relatively easily. In the USA, many resources have been prepared to prevent and control a potential plague bioweapon attack. The resources include, but are not limited to, pre-exposure measures, stockpiling antibiotics, and other post-exposure measures. However, to efficiently plan effective countermeasures, Hinckley et al. (2011) discuss that a better understanding of the transmission dynamics of pneumonic plague is needed. The authors found that the previous assumptions on transmission dynamics relied on data from large outbreaks, which inflate the estimate for the basic reproduction number R0. R0 represents the number of expected secondary infections introduced by a single infected person into a fully susceptible population if no public health countermeasures are taken. Instead of only large outbreaks, the authors used all available data on pneumonic plague cases in the USA from 1900 to 2009. They found that in most cases transmission failed, even in the absence of antimicrobial treatment or prophylaxis, which means that the actual R0 is nearly 1.0. For their study, Hinckley et al. (2011) used the notion of Rc, which is defined as the “average number of secondary cases generated by a single infectious case after control measures have been implemented.” The authors found that “in fact, all U.S. outbreaks during the pre-antibiotic era, including one having two reported super-spreading events, were controlled quickly and effectively with routine measures, as demonstrated by an estimated value for Rc of 0.76.” The measures consisted of “social distancing, isolation, quarantine, enhanced surveillance, contact tracing, and simple barrier precautions.” The authors conclude that since the most effective intervention may involve rapid identification and treatment of ill persons, decision makers should understand the likely transmission dynamics.

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