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Public Health Laboratories Ride a Funding Roller Coaster—
And It’s Dangerous

Scott J. Becker

Association of Public Health Laboratories, Silver Spring, Maryland

This year has been a roller coaster for the nation, especially for those of us in biosafety-related fields. At public health laboratories—the institutions charged with detecting and controlling diseases and other health threats—we’ve rolled and pitched through a major event, the novel H1N1 outbreak, and we know this crisis isn’t over yet. Yet it’s already obvious that we’re riding another cycle of health crisis followed by “fix-it-quick” policy. Even in the best of times, this recurring cycle never comes close to solving the systemic problems that challenge public health laboratories.

The irony is that while America depends on our work, we can’t depend on our funding. When we started 2009, we had hopes that public health labs might benefit from federal measures to boost the economy. But the stimulus package didn’t stimulate us. Then came novel H1N1. Lab workers, some called back from furloughs, worked 7 days a week accessioning, preparing, and testing specimens and then reporting test results.

Within weeks of the initial outbreak, 65 local and state public health laboratories were able to begin conducting confirmatory testing. This meant more work for our already overworked labs but greater safety for the public, thanks to the strong network built among the labs and the CDC (“Public Health Laboratories to Assume Primary Responsibility for Confirmatory Testing of Swine Flu,” APHL, April 30, 2009).

But meanwhile, back at the labs, staff was dealing with issues stemming from chronic under-funding:

• One lab, ready with trained staff and the right platform to perform the CDC’s five-target influenza assay, started testing—and the only instrument capable of running the tests promptly broke.
• Another lab had to perform manual testing while it waited for a software upgrade.
• Labs were training flu staff in the midst of the surge.
• Half a dozen public health labs did not have the required ABI 7500 FAST platform—and no way to acquire one. The reason? Budget constraints.
• Some labs were sending results to the CDC by fax.

In addition to H1N1 response activities, public health laboratories had to fulfill all their usual duties, from newborn screening to food-borne outbreak testing. (One such outbreak hit during the worst of the spring H1N1 crisis.)

What was the nation’s response? $1.5 billion in emergency funding through a supplemental appropriation (Letter from the President on Supplemental Appropriations Act, 2009 [Public Law 111-32]). Yes, this funding was urgently needed, and $1.5 billion is a hefty sum. But so far it appears the nation’s labs will receive no more than $20 million of this.

As those in the biosafety field know, rushing and overtaxing labs can compromise worker safety. During the height of the H1N1 crisis, many worked 12- and 16-hour shifts for days. When not enough employees were available to rotate through, some labs brought in temporary workers, training them on the fly. It was a heroic effort, and it succeeded, but it’s not one we want to have to try again.

Professional organizations carry the responsibility for setting standards, credentialing, and certifications in the areas of biosafety and biosecurity, but it’s those at the laboratory bench who pay the freight, in time and money, for the training needed to meet them. While the Association of Public Health Laboratories (APHL) is working to develop systems assessments, offer more training, and extend resources and partnerships to make the labs safe and high-quality, we can stretch our capacities only so far. For instance, we are currently working on distance modalities for training because travel budgets are strapped. But some instruction must be done hands-on.

Maintaining the physical infrastructure of our labs matters, of course. Add to this the challenge of keeping up with fast-changing technology, and you’re looking at several lines converging on the risk chart.

One of the most serious holes in our infrastructure is in reporting—public health labs simply do not have the rapid reporting systems needed to respond effectively in a surge event. Most submit test results using outmoded, labor-intensive methods: e-mail, fax, phone, and the CDC’s DOS-based system. Doctors and health decision makers rely on laboratory data to determine patient treatment and disease control measures, so any delay in delivery of lab data is detrimental to public health.

Laboratories are preventive health institutions. Electronic reporting expedites delivery of treatment and pub-
lic health response by accelerating delivery of data to decision makers. If the data lag, so does the intervention.

To address this urgent need to communicate with the CDC, APHL’s Public Health Laboratory Interoperability Project (PHLIP) aims to create a nationwide system for electronic exchange of lab data. Currently, through this CDC-supported project, five state labs can report test results electronically to the CDC, and more states are developing this capability. But sustained funding would get more labs on board faster.

A third rising area of risk is the overall lab workforce shortage. Getting more people into the profession is something both APHL and ABSA are working toward. Yet this, also, depends on sustained funding. If the jobs aren’t there or the jobs don’t pay, our encouragement won’t make a difference. No offense to plastic surgeons, but one can’t blame students for finding working with Botox more fiscally appealing, as it stands now, than working with anthrax when they contemplate their futures in science and medicine.

Those in biosafety know that you can’t plan for threats that aren’t yet known, and you can’t prepare for every threat. All we can do is have the best systems in place to meet threats when they get here, based on what we’ve learned from past events.

Preparedness starts well in advance of a crisis. For laboratorians, it’s not simply performing a drill or an exercise. Preparedness means staffing, training, and improving essential infrastructure. We experienced what being prepared was like early in this decade, when funding was available to meet biological and chemical threats. We’re still benefiting from the forward strides we made then—in creating partnerships, in renovating crumbling labs nationwide, in advancing our knowledge base.

Continuing on the crisis-funding roller coaster ignores the realities of the world in which we operate and the lessons we’ve learned. It’s a short-sighted approach with which anyone in science and medicine would take issue. As we in the labs know, safety starts with prevention, and it’s far better to avert a crisis than to respond to one. Let’s stop leveraging the crisis du jour and commit to steady funding of our laboratory first responders, the nation’s public health laboratories.

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**WHO Guidance Document—Updated and Revised**

The WHO has provided an updated and revised guidance document entitled “Laboratory Biorisk Management for Laboratories Handling Human Specimens Suspected or Confirmed to Contain Influenza A (H1N1) Causing the Current International Epidemics” as of November 30, 2009. This document is available on the web and has been posted at: www.who.int/csr/resources/publications/swineflu/LaboratoryHumanspecimensinfluenza/en/index.html

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**Responsible Research with Biological Select Agents and Toxins**

*Responsible Research with Biological Select Agents and Toxins* makes recommendations on the appropriate security measures for laboratories that conduct research using biological select agents and toxins (BSAT), a list of more than 80 dangerous pathogens that pose extreme public health and security risks. The report from the National Research Council (NRC) addresses both physical security and personnel reliability measures developed to protect against external and internal threats and focuses on those policies and practices that will be most effective and that minimize unintended consequences on the ability to conduct vital scientific research on BSAT. Available at: www.nap.edu/catalog.php?record_id=12774#
Investigation of the Benefits of Using Direct Steam Injection in Effluent Treatment Systems

Diane Gordon, Jay Krishnan, Les Wittmeier, and Steven Theriault
Public Health Agency of Canada, Winnipeg, Manitoba, Canada

Abstract

In high-containment facilities the treatment of biological waste is very important. Liquid waste from containment level 4 laboratories and containment level 3 laboratories which handle nonindigenous animal pathogens is collected and treated in effluent treatment vessels. These vessels decontaminate the effluent using indirect steam via a steam jacket to heat the liquid to a minimum of 121°C. In some containment facilities the effluent is decontaminated by using direct steam injection to heat the load to the decontamination set point. This method of injecting steam directly into the load has the potential of providing agitation and reducing the amount of time necessary to heat the load to the set point, thereby reducing the processing time and increasing system capacity.

To investigate the benefits of direct steam injection, one of the effluent treatment vessels was modified so that direct steam could be used to supplement the indirect heating of the effluent. After functional testing was conducted to ensure the proper operation of the steam injection, tests were conducted to determine the efficacy of decontamination. For these tests the liquid load was spiked with bacterial spores and samples were taken during the warm-up process as well as during the decontamination period to determine when inactivation of the spores was achieved. To date, very little data have been published on the efficacy of effluent treatment vessels or comparing different methods of heating in these vessels.

Introduction

In recent years research and diagnostics of emerging infectious diseases and agents of bioterrorism has increased, which has resulted in a rapid expansion in the number of high-containment facilities in the world. Due to the increased risk of the organisms handled in high-containment laboratories, increased safety protocols are required before waste from these facilities can be released into the public waste stream.

All waste coming out of containment laboratories must be decontaminated; this includes biohazardous waste, garbage, and liquid (effluent) waste. Large volumes of liquid waste may be generated due to the chemical and personal showers necessary upon exiting high containment. Liquids that drain from sinks, showers, autoclave chambers, and from washing down animal cubicles also contribute to the amount of liquid waste that must be treated before being released into the public sewer system.

Standards and guidelines in Canada require that all containment level 4 laboratories and containment level 3 laboratories which handle nonindigenous animal pathogens must have liquid effluent decontamination systems (Public Health Agency of Canada, 2004). International rules also stipulate the need for effluent treatment systems in high-containment laboratories (Mani & Langevin, 2006; Palani, 2006).

Effluent decontamination systems can utilize thermal decontamination, chemical decontamination, or a combination of the two, with thermal being the most common form. Thermal decontamination parameters vary based on the targeted microorganisms and the local regulations with which the facility must comply. Temperatures can range from 90°C-141°C with treatment times from 20 minutes to 2 hours (Mani & Langevin, 2006).

Thermal effluent treatment systems vary in design and operation. Effluent waste from containment laboratories can be collected in a receiving vessel later to be transferred to the treatment vessel or can be collected directly into a series of redundant treatment vessels. Decontamination can occur in a batch process or by continuous flow decontamination (Edwards, 2002; Mani & Langevin, 2006). During a general effluent decontamination cycle, the temperature of the effluent is raised to a set point and held at this temperature or above, for the specified period of time, after which the treated waste is cooled before releasing it into the municipal sewage system (Mani & Langevin, 2006; Palani, 2006).

Thermal effluent treatment vessels are generally heated to a set point using steam. Steam can be injected into a steam jacket located between the inner shell and the outer jacket of the vessel, utilizing the entire shell wall as a heating surface (Palani, 2006). This method is commonly known as indirect steam heating. Effluent treatment vessels can also be heated by inject-
ing steam directly into the liquid load. Direct steam injection has the advantages of more rapid heating and agitation of the liquid (Palani, 2006). The aim of this study was to investigate the benefits and efficacy of an effluent treatment vessel employing indirect heating supplemented with direct steam injection to heat effluent waste, while comparing it to the current system which employs only indirect steam heating. As part of this study, a protocol to validate the efficacy of effluent treatment systems was developed and experiments were conducted to determine the heat resistance of potential organisms for use in the validation protocol.

Materials and Methods

Heat Inactivation of Spores

Heat resistance was compared for three species of Bacillus spores: Bacillus thuringiensis (the spores used to test the efficacy of the effluent treatment system), Bacillus atropheaus ATCC 51189 (an organism commonly used in biological indicators for dry heat), and Bacillus anthracis ATCC 4229 (representing the most resistant pathogenic organism in high-containment laboratories), as well as Geobacillus stearothermophilus (an organism commonly used in biological indicators for wet heat). Given that Bacillus thuringiensis spores are not commonly used as a biological indicator, these experiments were conducted to compare the heat resistance of the above-mentioned bacterial spores to ensure that Bacillus thuringiensis spores are appropriate for testing the efficacy of the effluent treatment system.

Bacillus atropheaus ATCC 51189 and Bacillus anthracis ATCC 4229 spores were produced by inoculating organisms in sporulation media (10% Columbia broth with 0.1% MnSO₄ solution [10mM] in saline) and incubating for 72 hours at 37°C in an orbital shaker at 150 rpm. The spore suspension was washed three times by centrifugation and then resuspended in 1/10 the original culture volume of deionized distilled water. The spore suspension was then heat shocked at 80°C for 10 minutes to kill any vegetative bacteria (Springthorpe & Sattar, 2005). The concentration of spores was determined by filtering serial dilutions and adjusted as necessary. Dipel 2x biological pesticide (Valent Biosciences Corporation, Walnut Creek, CA) was used for Bacillus thuringiensis spores. The concentration was determined by resuspending the spores in sterile water and filtering serial dilutions. Spores of Geobacillus stearothermophilus were obtained from Prospore ampoule biological indicators (Raven Laboratories, Omaha, NE). Prospore ampoules were opened and the contents transferred to a centrifuge tube; spores were pelleted by centrifugation and resuspended in deionized distilled water. The concentration of spores was determined by filtering serial dilutions and adjusted as necessary.

Spore heat resistance was first tested using a Hira-yama stand-alone autoclave, model HV110 (Kasukabe-shi, Saitama, Japan). The minimum temperature and time exposure on this autoclave is 105°C for 1 minute. Spore heat resistance of the three Bacillus species was tested using spore suspensions and dried spores. For suspension tests, 1 ml containing 10⁶ spores in a microcentrifuge tube was tested in the autoclave. For dried spore tests, spores (10 ul containing 10⁶ spores) were dried on stainless steel carriers, transferred to Teflon vials, and placed in the autoclave. The autoclave cycle was run at 105°C with an exposure time of 1 minute. The cycle was immediately aborted after the 1-minute exposure and samples were removed. Dried spore samples were eluted in saline by vortexing. Samples were serially diluted in saline, plated on trypticase soy agar using vacuum filtration, and incubated at 37°C for bacterial growth. Growth was observed for 7 days, with initial colony counts taken after 24 hours of incubation. Spores of Geobacillus stearothermophilus are known to be very resistant to wet heat, so experiments were initially conducted at 121°C. A spore suspension in deionized distilled water at a total concentration of 10⁶ spores was autoclaved in a glass test tube at 121°C for 2, 4, and 8 minutes. Each time point was conducted separately and the autoclave cycle was immediately aborted after exposure. Samples were immediately removed, serially diluted in saline, plated on trypticase soy agar using vacuum filtration, and incubated at 56°C for bacterial growth. Growth was observed for 7 days, with initial colony counts taken after 24 hours of incubation.

Spore heat resistance of the three Bacillus species was also tested using a PCR thermocycler, Mastercycler EP Gradient S (Eppendorf, Westbury, NY). The thermocycler heats tubes from the bottom as well as the top ensuring even heating. The thermocycler heating block and lid were allowed to reach a temperature of 99°C and then PCR tubes containing 100 ul of spore suspension, at a concentration of 10⁶ spores/100 ul, were placed in the heating block and the lid was closed. After exposure time points, the samples were removed from the thermocycler. Samples were serially diluted in saline, plated using vacuum filtration, and incubated at 37°C for bacterial growth. After incubation of 24 hours, colonies were counted to determine the reduction in bacterial spores. Growth was observed for 7 days to monitor any changes in colony counts. All tests were carried out in triplicate. Colony counts were graphed and used to calculate the decimal reduction value (D-value). The D-value is the amount of time needed to inactivate 90% or 1 log₁₀ of the microbial population.

Effluent Treatment System

The effluent treatment vessels are jacketed horizontal cylindrical vessels with a neck fitted midway to accommodate filling, venting, and other requirements. The steam injection piping was inserted from the neck and consists of two offset eductors pointing in opposite directions towards the ends of the vessel (Figure 1). The
work also involved the addition of piping, control valves, and revised control logic.

Testing was conducted to ensure the proper operation of the direct steam injection; this consisted of ensuring that the vessel shell was adequately warmed up prior to initiating direct steam injection and that the rate at which the steam injection valve opened would not create severe turbulence within the vessel. A vibration switch was mounted on the vessel base and was programmed to turn off the steam injection if excess vibration was detected. A 10-point temperature probe was inserted from the neck to test for temperature stratification (Figure 1). This probe was used to monitor the temperature at 10 different locations during the effluent treatment cycle to ensure all areas of the vessel were above the decontamination set point. Also seen in Figure 1 is the location of the dual element probe which takes an average temperature and is used to control the process. A sampling port on the vessel is in close proximity to the dual element probe to allow sampling throughout the treatment cycle (Figure 1).

**Effluent Treatment Efficacy Testing**

Efficacy testing was conducted using Dipel 2x, a biological pesticide composed of spores of *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1. The dry spore powder was dissolved in sterile water and the concentration was determined to be $5 \times 10^{10}$ spores/gram. Efficacy testing was conducted using the current method of indirect steam heating and the new method using indirect steam heat supplemented with direct steam injection. For each method, tests were conducted three times on

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**Figure 1**

Schematic and pictures of the effluent treatment vessel.

A. Front and side view schematic of the effluent treatment vessel: (a) sampling port; (b) dual element temperature probe for controlling the process; (c) 10-point temperature probe for heat stratification tests' (d) entry point for spore solution; (e) entry point for water

B. Inside view of the effluent vessel after modifications for steam injection: (a) steam educators

C. Front view of the effluent vessel: (a) location of dual element temperature probes; (b) location of sampling port
separate days. In general, effluent treatment vessels are heated to a decontamination set point of 122°C. The temperature is monitored by the dual element probe, once the set point of 122°C is reached; a minimum temperature of 121°C is maintained for 30 minutes.

The vessel was filled with approximately 3500 ±10 litres of potable water; spore powder was added to the vessel during filling at 1500 L and 3000 L. After filling, a quick shot of steam was given to agitate the load and ensure a homogenous mixture. This yields a calculated final concentration of $10^6$ spores/ml of effluent. At this time, a sample was taken as a positive control to determine the starting concentration of spores. The liquid load was then heated to the decontamination set point of 122°C, with samples taken every 12 minutes during the warm-up phase. Once a temperature of 122°C was reached, a minimum temperature of 121°C was maintained for 30 minutes. Samples were taken every 2 minutes during the 30-minute decontamination phase. Prior to taking each sample, the sampling port was purged to ensure samples were taken directly from the vessel.

After completion of the decontamination phase, samples were taken to the laboratory for further processing. Samples were inoculated onto trypticase soy agar (TSA) plates in a biological safety cabinet using aseptic technique. Samples were incubated at 37°C and observed daily for bacterial growth for 7 days. The concentration of surviving spores was determined for samples that were positive for growth. For each positive sample, 1 ml of sample was serially diluted 10-fold in sterile saline. These dilutions were then plated on TSA using vacuum filtration. For samples showing no growth, 10 ml of sample was plated on TSA using vacuum filtration. Colonies were counted after incubation at 37°C for 24 hours to determine the $\log_{10}$ reduction by comparing colony counts of the control. Growth was observed for 7 days to monitor any changes in colony counts.

Results

Heat Inactivation of Spores

*Bacillus thuringiensis*, *Bacillus atrophaeus*, and *Bacillus anthracis* spores were completely destroyed in the autoclave. No viable spores could be detected in either the spore suspensions or dried spores of all three species after autoclaving at 105°C for 1 minute. *B. atrophaeus*, *B. anthracis*, and *B. thuringiensis* exhibited the same level of resistance to heat using an autoclave at 105°C for 1 minute. *Geobacillus stearothermophilus* was much more resistant to inactivation by autoclaving. A reduction of greater than 6 logs was observed after autoclaving at 121°C for 4 minutes. A D-value of 0.6 minutes was calculated for *G. stearothermophilus* when autoclaving at 121°C.

A steady decline in viable spores was observed for all three species tested when exposed to heat using a thermocycler at 99°C. Starting spore concentrations were between 5 and 6 logs, slightly lower than antici-

Figure 2

Spore counts ($\log_{10}$) for the three *Bacillus* spore species exposed to 99°C in a thermocycler for 1, 2, and 3 minutes compared to starting concentrations.
pated. For all three species, a 3-minute exposure to 99°C resulted in a 5-log reduction in viable spores. As seen in Figure 2, the number of viable spores declined more rapidly for *B. anthracis* than the other two species tested. Calculated D-values at 99°C for *B. anthracis*, *B. atropheaus*, and *B. thuringiensis* were 0.6, 0.7, and 0.6 minutes, respectively. The results of these experiments show that *Bacillus thuringiensis* spores have a similar resistance to heat as *Bacillus anthracis* and *Bacillus atropheaus* and are considered acceptable for use in the efficacy testing of heat inactivation.

**Effluent Treatment Efficacy Testing**

For all three trials conducted using both heating methods, no viable spores could be detected in samples taken at the completion of the decontamination phase, indicating both methods were effective in inactivating *Bacillus thuringiensis* spores. Effluent treatment using only indirect steam heating resulted in an average warm-up phase of 104 ± 4.00 minutes. During the warm-up phase, a decline in the number of viable spores was observed. For two of the trials, a complete inactivation of spores was observed at 36 minutes into the warm-up phase and no viable spores could be detected after this point. For the third trial, a complete inactivation of spores was observed 60 minutes into the warm-up phase; no viable spores were detected after this point. A graphical representation of the temperature and spore concentration during the treatment cycle can be seen in Figure 3.

The addition of direct steam injection resulted in a much shorter average warm-up phase of 45 ± 2.52 minutes. During the warm-up phase, a steady decline in the number of viable spores was detected for all three trials. A graphical representation of the decline in viable spores during the treatment cycle can be seen in Figure 4. In the first trial, a complete inactivation of spores was observed at 44 minutes into the treatment cycle, which was 1 minute after the effluent reached the decontamination temperature of 122°C. In Trials 2 and 3, the decontamination temperature was reached a few minutes later than in Trial 1. A complete inactivation of spores was observed 36 minutes into the warm-up phase for both Trials 2 and 3 at an average temperature of 104°C.

As seen in Figure 5, both heating methods achieved a complete inactivation of spores in a similar amount of time. The major difference observed was the amount of time required to reach the decontamination set point. Using indirect heat supplemented with direct steam injection resulted in an average warm-up time of 45 minutes, while using only indirect heat required more than twice that amount of time (Figure 5).

Results from the temperature stratification tests can be seen in Figure 6. Probes 1-10 correspond to the 10 points on the temperature probe, locations of which can be seen in Figure 1. Throughout the 30-minute decontamination phase, the 10 probes recorded temperatures well above the decontamination set point of 122°C, with an average temperature of approximately 135°C. As seen in Figure 6, the dual element probe which con-
**Figure 4**
Trial 2: Temperature and *Bacillus thuringiensis* spore counts (log_{10}) over time during effluent treatment using direct steam injection. Spore counts decreased as the temperature of the effluent increased. Three trials were conducted, exhibiting similar results. (▲) Temperature (●) Spore Counts

**Figure 5**
The amount of time required to reach sterilization set point (122°C) and to achieve a complete kill for the two effluent vessel heating methods.

**Comparison of Heating Methods**

- [Time to Reach Sterilization Set Point]
- [Time to Reach Spore Kill]
trols the process recorded lower temperatures during the decontamination phase. The temperature stratification results using direct steam injection were similar to that previously conducted using only indirect heating.

Discussion

Effluent treatment processes must be validated at the commissioning of a facility or when significant changes to the process are made in order to ensure that biological decontamination of more than $10^6$ microorganisms is achieved. This is referred to as the “sterility assurance level,” as the chances of a micro-organism surviving is one in a million (U.S. Department of Health and Human Services, 2007). The organism most commonly used for validating wet thermal decontamination is *Geobacillus stearothermophilus*, as spores of this organism are extremely heat resistant (Edwards, 2002). Spores of *Bacillus atropheaus*, formerly known as *Bacillus subtilis*, are resistant to dry heat and many other decontamination methods and are often used in biological indicators to validate decontamination methods (Wittmeier, 2004).

Very little published data exist on efficacy testing and the validation of effluent treatment systems. One published study used glass vials of *Geobacillus stearothermophilus* spores placed in a long tube inside the treatment vessel (Edwards, 2002). Direct inoculation of the effluent with the test organism was the testing method chosen for this study, as it best represents the environment infectious organisms in high-containment effluent would experience. In this method, the test organism is fully submerged in the liquid effluent, unlike the method using vial spores where the test organism is separated from the effluent by a glass vial. One problem encountered with direct inoculation of the effluent is that growing spores of *Geobacillus stearothermophilus* or *Bacillus atropheaus* to the high concentrations necessary for efficacy testing of such large loads is difficult and can be quite costly. This is often the reason why vial spores are chosen as a validation method over direct inoculation (Edwards, 2002). *Bacillus thuringiensis* spores were investigated for use in effluent treatment efficacy testing, as they were easily acquired at a high concentration in the form of a dry biological pesticide. Given that *Bacillus thuringiensis* spores are not com-

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Figure 6

Temperature stratification tests of the effluent treatment vessel using indirect heating supplemented with direct steam injection. Temperature readings during the 30 minutes decontamination phase of the 10 probes, the average of the 10 probes and the average temperature of the dual element probe used to control the process.
monly used for the validation of decontamination, experiments were conducted to compare the heat resistance of spores of *Bacillus thuringiensis* with *Bacillus atrophaeus*, *Bacillus anthracis*, and *Geobacillus stearothermophilus*.

Experiments conducted using an autoclave to compare the heat resistance of the three Bacillus spore species resulted in complete inactivation of the three species tested. It is important to note that although the spores were exposed to 105 °C for only 1 minute, they were exposed to heat for much longer, as one must include the amount of time required to heat the autoclave to the set point. It is likely that this warm-up time greatly contributed to the inactivation of the spores. Results from these experiments lead one to believe that the effluent treatment cycle should also be successful in completely inactivating Bacillus spore species. The effluent treatment system has a higher set point temperature (121 °C compared to 105 °C) and a longer exposure time (30 minutes compared to 1 minute) in comparison to the autoclave tested. As expected, *Geobacillus stearothermophilus* was much more heat resistant and a D-value of 0.6 minutes was calculated using the autoclave at 121 °C. This method of heat inactivation did not provide the necessary data to compare the heat resistance of the three Bacillus spore species in question, as complete inactivation of all three species was found at the lowest possible temperature and exposure time. Also, it is not possible to place the spores in the autoclave after the sterilization set point is reached to exclude any inactivation during the autoclave warm-up phase. Due to the limitations of the autoclave, another testing method at a lower temperature that does not incorporate the warm-up phase was necessary.

The method of using a thermocycler to test the heat resistance of bacterial spores was chosen, as this method allows testing at a lower temperature of 99 °C and also allows exposure of the spores to heat once the temperature set point was reached. This method eliminates the potential for inactivation during the warm-up phase. Data from these experiments indicate the three Bacillus spore species tested have similar resistance to heat, with D-values of 0.6 to 0.7 minutes. Based on these findings, *Bacillus thuringiensis* was considered an acceptable surrogate to *Bacillus anthracis* for efficacy testing of heat inactivation.

Although *Geobacillus stearothermophilus* spores are the most resistant to wet heat, spores of *Bacillus anthracis* represent the organism most resistant to decontamination that would be encountered in the effluent waste of a high-containment laboratory. The general order for micro-organisms’ resistance to decontamination, beginning with the most resistant, is bacterial spores, mycobacteria, naked viruses, fungi, vegetative bacteria, and enveloped viruses (U.S. Department of Health and Human Services, 2007). Based on this pattern, an effluent treatment system that is effective in inactivating *Bacillus anthracis* is also effective in inactivating other pathogens manipulated in high-containment laboratories. These include, *Mycobacterium tuberculosis*, Foot-and-Mouth disease, *Yersinia pestis*, and Ebola virus. Therefore, a bacterial spore exhibiting similar heat resistance to *Bacillus anthracis*, such as *Bacillus thuringiensis*, is considered an acceptable surrogate for the efficacy testing and validation of effluent treatment systems.

The effluent treatment efficacy studies conducted demonstrate that both indirect steam injection and a combination of direct and indirect steam injection were effective in achieving inactivation of *Bacillus thuringiensis* spores. The major benefit of adding direct steam injection, observed during this study, was a decrease in the amount of time required to reach the decontamination set point. The amount of time required to reach the set point was twice as long when using only indirect heat in comparison to direct steam injection in conjuction with indirect heating. A reduction in the warm-up phase resulted in a shorter overall cycle, allowing the effluent vessel to accept another load of waste in a shorter period of time. A shorter cycle in each vessel greatly increases the overall effluent treatment system capacity. In a busy high-containment facility, an increase in effluent treatment system capacity is very important, as it ensures the system is ready to handle liquid waste at all times resulting in the reduced likelihood of interruptions for the program staff.

Temperature stratification tests demonstrate that all areas of the vessel are reaching and maintaining a temperature above the decontamination set point for the duration of the cycle. The dual temperature probe that controls the cycle recorded the lowest temperature and samples were taken at this location.

Direct steam injection also provided agitation to the effluent load. This can help to ensure equal heating throughout the load (Palani, 2006). This is a much simpler method to provide agitation compared to internal mixers which would have added installation and maintenance costs. Another potential benefit of using direct steam injection is the ability to use the steam to clean the inside of the vessels. This potential benefit requires further investigation.

In conclusion, this study has provided a realistic method for testing the efficacy of effluent treatment systems. Spores of *Bacillus thuringiensis* in the form of a dry biological pesticide provide a convenient testing method. These spores have a similar heat resistance to *Bacillus anthracis* based on the D-values obtained at 99 °C. The effluent treatment system utilizes a much higher temperature (>121 °C) and a much longer exposure time (30 minutes) than the conditions used for heat-resistance testing, thus ensuring a successful inactivation of the spores. Also, the 10-point temperature probe indicated the decontamination set point was reached at all test points. The addition of direct steam injection to assist in the heating of effluent treatment
vessels does not significantly change the system protocols or introduce any complications. Direct steam injection provides several benefits to the overall process and is equal in efficacy to the currently used method.

Acknowledgments

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References


A Novel Approach for Conducting Room-scale Vaporous Hydrogen Peroxide Decontamination of Virulent Bacillus anthracis Spores

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Abstract

Studies have been conducted to determine the efficacy of various decontamination technologies against virulent B. anthracis and surrogate spores within small, bench-scale chambers. This study assessed an approach for evaluating room-scale (~2,700 ft³) decontamination efficacy of vaporous hydrogen peroxide fumigation against B. anthracis Ames and B. subtilis spores deposited onto porous and non-porous indoor surface materials. Approximately 1x10⁸ colony-forming units (CFU) of B. anthracis and B. subtilis spores were dried onto galvanized metal and ceiling tile coupons and then exposed to vaporous hydrogen peroxide. The materials contaminated with B. anthracis spores were placed inside a Class III biosafety cabinet (BSC III) that circulated vaporous hydrogen peroxide from within the decontaminated room, into and out of the BSC III. Identical materials inoculated in the same manner and at the same density with B. subtilis were placed both inside and outside of the BSC III to compare decontamination efficacy. Three fumigations were conducted using two sets of cycle parameters. The first set of cycle parameters for vaporous hydrogen peroxide exposure (10 minutes of conditioning at 12 g/min; 75 minutes of decontamination at 11 g/min) yielded log reductions in viable B. anthracis and B. subtilis spores ranging from 6.1 to 7.0 on all materials, while only 76% of the commercial biological indicators (1x10⁶ CFU) evaluated in parallel were completely inactivated. The second set of cycle parameters (12 minutes of conditioning at 12 g/min; 104 minutes of decontamination at 8 g/min) yielded log reductions in viable B. anthracis and B. subtilis spores ranging from 6.7 to 7.4 on all materials and complete inactivation of biological indicators. These results demonstrate this method as a viable approach to assess room-scale fumigant decontamination efficacy against B. anthracis Ames spores.

Introduction

Since the intentional release of Bacillus anthracis spores in 2001 in the United States, studies have been conducted to determine the efficacy of various decontamination technologies against B. anthracis spores, which have included liquids, gels, and fumigants. Of
these, fumigants (gases and vapors) are the preferred method for decontaminating large areas due to advantages in distribution and the ability to interact with large, complex surfaces. An industry standard for years has been formaldehyde fumigation. This decontamination approach is efficacious against *B. anthracis* spores on porous and non-porous materials (Rogers et al., 2007); however, when the formaldehyde is neutralized, it leaves behind a residue (hexamethylene tetramine) that requires additional clean-up efforts. Vaporous hydrogen peroxide, which was used to remediate the Hart Senate Suite in 2001, offers a cleaner, less toxic alternative to formaldehyde and is an accepted method for decontamination in the scientific, medical, and pharmaceutical communities (Anderson et al., 2006; Boyce et al., 2008; Dryden et al., 2008; Fichet et al., 2004; French et al., 2004; Heckert et al., 1997; Hillman, 2004; Johnston et al., 2005; Klapes & Vesley, 1990; Krause et al., 2001; Krishna et al., 2000; Otter & Budde-Niekiel, 2009; Otter & French, 2009; Otter et al., 2009; Rastogi et al., 2009; Rogers et al., 2009; Verce et al., 2008; Wagenaar & Snijders, 2004). These studies using vaporous hydrogen peroxide provide efficacy data over a wide range of operational and environmental conditions. However, there is a need to test and challenge the efficacy of fumigant decontamination technologies in larger room-scale environments against various biological agents since most studies conducted to date have been on smaller-scale environments.

Laboratory-controlled decontamination testing of vaporous hydrogen peroxide at room-scale has been conducted using surrogates for *B. anthracis* spores or other biological select agents (Hall et al., 2007; Kahnert et al., 2005; Otter & French, 2009). Using a 317 L chamber, vaporous hydrogen peroxide was shown in previous studies to be efficacious against *B. anthracis*, *B. subtilis*, and *G. stearothermophilus* spores in which statistical differences were observed between *B. anthracis* and surrogate spores on porous and non-porous materials (Rogers et al., 2005). The efficacy of vaporous hydrogen peroxide has also been demonstrated against two virulent strains and one avirulent strain of *B. anthracis* within a 15,000 L chamber (Rogers et al., 2009). However, potential challenges are associated with switching from a bench-scale approach to a room scale, such as controlling environmental conditions, material compatibility, and achieving sufficient fumigant concentrations necessary to inactivate microorganisms. Such complexities could potentially confound the utilization of efficacy data derived from bench-scale tests for making informed decisions regarding the decontamination of rooms or buildings. This prompts a growing need to test and generate more efficacy data using fumigant decontamination technologies in large room-scale environments. Therefore, the purpose of this study was to demonstrate a safe approach to conducting controlled room-scale fumigant decontamination studies to assess the efficacy of vaporous hydrogen peroxide against *B. anthracis* Ames spores.

### Materials and Methods

#### Test Organisms

All phases of testing were performed under biosafety level 3 (BSL-3) conditions. Virulent *B. anthracis* Ames spores were prepared in a fermentor as previously described (Rogers et al., 2005). The *B. subtilis* (ATCC 19659) spores were prepared using a shaker flask method as previously described (Rogers et al., 2006). The purified spore preparations were resuspended in sterile water and evaluated by phase-contrast microscopy. Preparations having >95% refractile spores with <5% cellular debris were enumerated, diluted to approximately 1x10⁹ CFU/ml and stored at 2º-8ºC.

Stainless steel biological indicators (BI) (Raven Labs, Omaha, NE) inoculated with ~1x10⁶ *B. atrophaeus* spores packaged in Tyvek were used to qualify the fumigant distribution and efficacy of the decontamination cycle parameters.

#### Test Materials and Chamber

Galvanized metal and ceiling tile test materials (1.9 cm x 7.5 cm) were selected to represent non-porous and porous surfaces, respectively. Each test material was inspected for defects and appearance before and after decontamination to aid in determining material compatibility with the fumigant. Additionally, 16 hydrogen peroxide chemical indicator (CI) strips NB305 (STERIS Corporation, Mentor, OH) were located throughout the room, including three located within the BSC III (Figure 1). These indicators were used to qualify a decontamination cycle within the enclosure by indicating exposure to hydrogen peroxide.

The experiments were conducted in a 2,727 ft³ (77.2 m³) BSL-3 laboratory. The room contained a 1,680 ft³ (47.6 m³) bioBubble® (The Colorado Clean Room Company, Ft. Collins, CO) possessing four HEPA-filtered blower units that maintained negative pressure with approximately 100 air exchanges per hour. The bioBubble® is a soft-walled enclosure constructed of 16 mil clear vinyl walls stretched over a 3.18 cm tubular aluminum frame. The vinyl walls connect using hook and loop seams. A 27 ft³ Class III biosafety cabinet (BSC III) (The Baker Company, Sanford, MA) was placed within the room and outside the bioBubble® which circulates vaporous hydrogen peroxide from the laboratory air into and out of the enclosure through HEPA filters at the rate of 100 cubic feet per minute (CFM). In addition to the four bioBubble® blowers, six circulation fans were placed throughout the room to aid in distribution of the vapor (Figure 1). Injection and return ports to the laboratory were not available; therefore, a Plexiglas “false door” was constructed to cover the doorway opening that was modified with ports to accommodate the inlet and return
lines to the Steris VHP® 1000 (Figure 1).

Test materials (N=3 per material) were inoculated with 100 µL of a 1x10⁹ CFU/ml suspension of B. anthracis or B. subtilis, resulting in ~ 1x10⁸ CFU/test material. This was accomplished using a multichannel pipette that dispensed two rows of five droplets (10 µL per droplet). These materials were allowed to dry within a BSC III for approximately 8 hours before initiating the decontamination cycle. Prior to decontamination, three test materials of each type were inoculated with B. anthracis and B. subtilis and corresponding un-inoculated blanks (N=1) were transferred into the test chamber BSC III. Three additional galvanized metal coupons inoculated with B. subtilis (and one blank) were placed adjacent to and outside the BSC III to compare a potential difference in decontamination efficacy between the laboratory room and the BSC III. A total of 50 BI were placed within the laboratory of which three were placed inside the BSC III (Figure 1). In parallel, three control coupons (inoculated, not decontaminated) and one blank (not inoculated, not decontaminated) of each material type and organism were transferred into a Plaslabs model 830-ABC compact glove box control chamber (Plaslabs Inc., Lansing, MI) that was maintained at ambient temperature and relative humidity for the duration of the decontamination cycle.

**Decontamination Procedure**

A Steris® series 1000 VHP® generating unit, which uses 35% hydrogen peroxide (Vaprox®; STERIS® Corporation) to generate the vapor within the target enclosure, was used to decontaminate the laboratory. This generator performs and controls four phases during the sterilization cycle: dehumidification, conditioning, decontamination, and aeration. During the dehumidification phase, the unit removes moisture from the enclosure to a defined set point prior to the injection of 35% hydrogen peroxide. The conditioning phase promotes a rapid in-
crease in hydrogen peroxide levels within the enclosure over a relatively short amount of time (e.g., 10 minutes). The decontamination phase has a reduced injection rate that maintains the vaporous hydrogen peroxide concentration (achieved during conditioning) over an extended period of time (e.g., 75 minutes). Aeration, in which the vaporous hydrogen peroxide is circulated through HEPA filters and a catalytic converter for neutralization, is initiated once the programmed decontamination time has concluded.

Three fumigations were conducted using two separate sets of cycle parameters (Table 1). The first set of cycle parameters was established by following the Cycle Development Guide for VHP® Biodecontamination System Products (STERIS Corporation). Cycle parameters for subsequent runs were adjusted based on data obtained from the first set of cycle parameters. Prior to the initiation of the decontamination cycle, the HVAC to the room was shut down to prevent the vaporous hydrogen peroxide from exhausting from the laboratory. Each run was initiated at the end of the workday and cycle parameters run in order as outlined in Table 1 and allowed to run overnight. The following morning the HVAC was re-opened and the room was allowed to conclude the aeration process to below 1 ppm hydrogen peroxide. These concentrations were measured using a low level Dräeger monitor (Dräeger Safety, Inc., Pittsburgh, PA). The temperature and relative humidity (RH) were monitored using a HOBO data logger (Onset Computer Corporation, Pocasset, MA).

Sample Processing
Following the aeration phase of the decontamination cycle, the test coupons, biological indicators, and chemical indicators were removed from the laboratory. The galvanized metal and ceiling tile test coupons were placed in 50 ml conical tubes containing 10 ml of sterile phosphate buffered saline (Sigma, St. Louis, MO) containing 0.1% Triton X-100 (Sigma). The spores were extracted via agitation at 200 rpm on an orbital shaker for 15 minutes at room temperature. Following extraction, 1.0 ml of each extract was removed and tenfold serial dilutions were prepared in sterile water. A quantitative assessment of each sample was determined by dilution plating where 100 µl of the undiluted extract and each serial dilution were plated onto tryptic soy agar (TSA) (Hardy Diagnostics, Santa Maria, CA) plates in triplicate, allowed to dry, and incubated for 18-24 hours at 37 °C. Following incubation, the plates were enumerated and CFU/ml was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution. Data were then expressed as the mean ± SD of the total CFU.

All biological indicators were aseptically transferred into 15 ml tubes containing 10 ml of tryptic soy broth (Remel, Lenexa, KS), gently agitated, and incubated at 37 °C. Samples were assessed for growth (turbid culture) or no growth (clear culture) at 1 day and 7 days. Chemical indicator strips were visually assessed for a color change, indicating the presence of hydrogen peroxide.

Results
Three decontamination runs were completed with vaporous hydrogen peroxide in which the decontamination phases included 85 minutes (run 1) or 116 minutes (runs 2 and 3) delivering approximately 945 g and 976 g of hydrogen peroxide, respectively. The total cycle time for each replicate run, including dehumidification, conditioning, decontamination, and aeration phases, totaled approximately 17 hours. From the initiation of the run, temperature and RH levels increased from 18.6°C and 38.5% RH to 33.9°C and 95.8% RH at the end of the decontamination phase. This increase in temperature may have resulted from the approximate 60°C delivery temperature of the vaporous hydrogen peroxide into the room in which the environmental controls for air flow and conditioning had been turned off. Temperature and relative humidity levels returned to normal conditions (~20°C and ~42% RH) following the aeration phase. Figure 2 provides a representative graph of temperature change.

Table 1
Vaporous Hydrogen Peroxide Decontamination Parameters

<table>
<thead>
<tr>
<th></th>
<th>Dehumidification</th>
<th>Conditioning</th>
<th>Decontamination</th>
<th>Aeration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow Rate (CFM)</td>
<td>H₂O₂ mg/L (%)</td>
<td>Duration (min)</td>
<td>Flow Rate (CFM)</td>
</tr>
<tr>
<td>Test Run #1</td>
<td>20</td>
<td>6.9 (30)</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Test Run #2</td>
<td>20</td>
<td>6.9 (30)</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Test Run #3</td>
<td>20</td>
<td>6.9 (30)</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

aCFM = cubic feet per minute
and relative humidity within the laboratory for the duration of each experimental run.

For all three decontamination runs, visual inspection of the CIs revealed that all 16 were positive for color change, indicating exposure to the vaporous hydrogen peroxide. The unexposed control CIs located in the anteroom exhibited no color change. For test 1, 12 out of 50 (24%) BIs were positive for growth at 1 day and 7 days (Table 2). Replicate tests 2 and 3 resulted in 0 out of 50 BIs exhibiting growth at 1 day and 7 days (Table 2). For all three decontamination runs, all positive control BIs exhibited growth; the negative control tubes exhibited no bacterial growth at 1 day and 7 days (Table 2). A sample of each positive test carrier was plated onto TSA to confirm the identity and homogeneity of the test organism.

Test materials contaminated with Bacillus anthracis Ames and exposed to vaporous hydrogen peroxide resulted in complete inactivation and log reductions ranging from 6.7 to 7.0 on ceiling tile and 7.0 to 7.1 on galvanized metal (Tables 3 and 4). Test materials contaminated with B. subtilis and exposed to vaporous hydrogen peroxide exhibited log reductions ranging from 6.1 to 7.4 on galvanized metal (inside BSC III), 6.6 to 7.4 on galvanized metal (outside BSC III), and 6.8 on ceiling tile (Tables 3 and 4). Viable test organisms were not recovered from any of the blank (uninoculated) test materials. Upon visual inspection, no physical damage was observed for the hydrogen peroxide-exposed coupons.

Discussion

This study provides a new approach for room-scale decontamination of biological select agents using vaporous or gaseous decontaminants. The inactivation data for B. anthracis Ames spores presented here resulted from an iterative process of establishing vaporous hydrogen peroxide decontamination cycle parameters for an approximately 2,700 ft³ laboratory. The method outlined in this study provides a safe and controlled approach to test whether or not established decontamination parameters are effective for inactivating biological agents on different types of material surfaces. Previously, room-scale or larger decontamination studies have targeted non-select agents, such as methicillin-resistant Staphylococcus aureus, Acinetobacter spp., Klebsiella pneumonia, Mycobacterium tuberculosis, Clostridium difficile, bacteriophages, and fungi (Andersen et al., 2006; Boyce et al., 2008; Dryden et al., 2008; French et al., 2004; Grare et al., 2008; Hall et al., 2007; Hall et al., 2008; Kahnert et al., 2005; Luftman et al., 2006; Otter & Budde-Niekel, 2009; Otter & French, 2009; Otter et al., 2009). In response to the 2001 anthrax attacks, the inactivation of B. anthracis spores by fumigants was performed by decontaminating spaces having volumes ranging from approximately 8,300 ft³ to 14 million ft³ (Canter et al., 2005). However, most of the laboratory testing of fumigant decontaminants against biological select agents (e.g., B. anthracis, Francisella tularensis, Yersinia pestis) has been conducted using bench-scale chambers ranging from approximately 300 L to 1,275 L (Rogers et al., 2005; Rogers et al., 2007; Rogers & Choi, 2008; Rogers et al., 2008).

In this study, the efficacy data from decontamination run 1 using the first set of cycle parameters (Table 1) show inactivation of both B. anthracis Ames and B. subtilis on all test materials inside the BSC III as well as B. subtilis on galvanized metal in the room (outside the BSC III) to a level of ≥6.1 logs. All of the CIs within
### Table 2
Biological and Chemical Indicator Results

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Biological Indicators (No. positive/No. tested)</th>
<th>Chemical Indicators (No. positive/No. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
</tr>
<tr>
<td>Test Run #1</td>
<td>12/50</td>
<td>12/50</td>
</tr>
<tr>
<td>Test Run #2</td>
<td>0/50</td>
<td>0/50</td>
</tr>
<tr>
<td>Test Run #3</td>
<td>0/50</td>
<td>0/50</td>
</tr>
</tbody>
</table>

### Table 3
Vaporous Hydrogen Peroxide Decontamination of *B. anthracis* and *B. subtilis* on Porous and Non-porous Surfaces Test Run 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Organism</th>
<th>Mean Total Recovered Spores ±SD (N=3)</th>
<th>Mean Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceiling Tile Control</td>
<td><em>B. anthracis</em></td>
<td>1.07 ± 0.22 x 10⁷</td>
<td>-</td>
</tr>
<tr>
<td>Galvanized Metal Control</td>
<td><em>B. anthracis</em></td>
<td>9.78 ± 0.33 x 10⁶</td>
<td>-</td>
</tr>
<tr>
<td>Ceiling Tile Decon</td>
<td><em>B. anthracis</em></td>
<td>0</td>
<td>≥ 7.03</td>
</tr>
<tr>
<td>Galvanized Metal Decon</td>
<td><em>B. anthracis</em></td>
<td>0</td>
<td>≥ 6.99</td>
</tr>
<tr>
<td>Ceiling Tile Control</td>
<td><em>B. subtilis</em></td>
<td>6.24 ± 3.57 x 10⁶</td>
<td>-</td>
</tr>
<tr>
<td>Galvanized Metal Control</td>
<td><em>B. subtilis</em></td>
<td>4.04 ± 0.32 x 10⁶</td>
<td>-</td>
</tr>
<tr>
<td>Ceiling Tile Decon</td>
<td><em>B. subtilis</em></td>
<td>0</td>
<td>≥ 6.80</td>
</tr>
<tr>
<td>Galvanized Metal Decon</td>
<td><em>B. subtilis</em></td>
<td>0</td>
<td>≥ 6.10</td>
</tr>
<tr>
<td>Galvanized Metal Decon (outside BSC III)</td>
<td><em>B. subtilis</em></td>
<td>0</td>
<td>≥ 6.61</td>
</tr>
</tbody>
</table>

### Table 4
Vaporous Hydrogen Peroxide Decontamination of *B. anthracis* and *B. subtilis* on Porous and Non-porous Surfaces Test Runs 2 and 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Organism</th>
<th>Mean Total Recovered Spores ±SD (N=6)</th>
<th>Mean Log Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceiling Tile Control</td>
<td><em>B. anthracis</em></td>
<td>6.51 ± 3.93 x 10⁶</td>
<td>-</td>
</tr>
<tr>
<td>Galvanized Metal Control</td>
<td><em>B. anthracis</em></td>
<td>1.75 ± 1.36 x 10⁷</td>
<td>-</td>
</tr>
<tr>
<td>Ceiling Tile Decon</td>
<td><em>B. anthracis</em></td>
<td>0</td>
<td>≥ 6.74</td>
</tr>
<tr>
<td>Galvanized Metal Decon</td>
<td><em>B. anthracis</em></td>
<td>0</td>
<td>≥ 7.10</td>
</tr>
<tr>
<td>Ceiling Tile Control</td>
<td><em>B. subtilis</em></td>
<td>5.68 ± 1.21 x 10⁶</td>
<td>-</td>
</tr>
<tr>
<td>Galvanized Metal Control</td>
<td><em>B. subtilis</em></td>
<td>2.95 ± 1.75 x 10⁷</td>
<td>-</td>
</tr>
<tr>
<td>Ceiling Tile Decon</td>
<td><em>B. subtilis</em></td>
<td>0</td>
<td>≥ 6.75</td>
</tr>
<tr>
<td>Galvanized Metal Decon</td>
<td><em>B. subtilis</em></td>
<td>0</td>
<td>≥ 7.39</td>
</tr>
<tr>
<td>Galvanized Metal Decon (outside BSC III)</td>
<td><em>B. subtilis</em></td>
<td>0</td>
<td>≥ 7.39</td>
</tr>
</tbody>
</table>
the BSC III changed color, indicating positive exposure to vaporous hydrogen peroxide. These results suggest: (1) the vaporous hydrogen peroxide introduced into the room is being circulated from the room into the BSC III as intended; and (2) the relative concentration of vaporous hydrogen peroxide in the room and BSC III yielded comparable levels of spore inactivation. However, for this first run complete inactivation of all BIs (Table 2) in which the BIs exhibiting positive growth were located at positions within the room that were expected to possess reduced or minimal air circulation (see locations in Figure 1), such as on the wall behind the laboratory door (BIs #2, 5), in between the bioBubble® and the wall/ceiling (Bls 15, 26), on the inside wall of the bioBubble® (Bls #28, 31, 34, 35), inside a bioBubble® exhaust flue (Bl #18), and in and around the BSC III (Bls #44, 46, 49), was not achieved. Since most of the BIs yielding positive growth were in close proximity to the bioBubble®, possibly an additional factor contributing to this lack of BI inactivation could be due to absorption of vaporous hydrogen peroxide by the large plastic covering of the bioBubble®. This could potentially lead to a decrease in the local and total room concentration of vaporous hydrogen peroxide, thus affecting decontamination efficacy. When switching from laboratory-scale to room-scale testing, factors that could impact the final efficacy results must be considered. These can include controlling temperature, relative humidity, ability to seal the room, achieving/maintaining target decontaminant concentration, and the complexity of the structural materials. Material compatibility issues need to be considered as vaporous/gaseous decontaminants can partition into or react with different materials within the containment space, thereby reducing the available decontaminant concentration and ultimately affecting microbial inactivation. Such partitioning or potential reaction with materials by vaporous hydrogen peroxide has been described previously for Plexiglas® (Baron et al., 2007) and metal ductwork (Verce et al., 2008). Therefore, based on the findings from decontamination run 1, adjustments to the cycle parameters were made to extend the initial injection time of hydrogen peroxide from 10 minutes to 12 minutes, lower the decontamination injection rate from 11 g/min to 8 g/min, and extend the overall contact time (conditioning plus decontamination) from 85 minutes to 116 minutes. The changed parameters were confined to the limits of the VHP® 1000 capacity of approximately 1,000 g of total hydrogen peroxide delivery. The subsequent two runs implementing these adjustments resulted in the inactivation of spores inoculated on test materials and complete inactivation of all BIs throughout the room and inside the BSC III, indicating the second set of decontamination parameters were the most efficacious.

Fumigation technologies often rely on theoretical calculations for determining the final decontaminant concentration within the targeted fumigation space without directly measuring the decontaminant to verify or confirm these values. This can be problematic when conducting decontamination runs in large spaces filled with different material types (e.g., plastics), spaces that cannot be completely sealed (e.g., HVAC exhaust), or chemicals that break down in the presence of various environmental conditions (e.g., chlorine dioxide degradation in light). The VHP® 1000 used in this study does not monitor vaporous hydrogen peroxide concentrations in real time; this decontamination system injects a known quantity of hydrogen peroxide over a specified length of time. The ability to monitor and maintain fumigant concentrations throughout a decontamination cycle seems to be a critical component to achieving consistent biological inactivation. This is especially important when potential material compatibility issues reduce actual concentrations compared to theoretical calculations. One important factor in implementing the decontamination approach, described in this study using a recirculating BSC III inside a laboratory undergoing fumigation, is determining whether the decontaminant concentration in the BSC III is similar to or equivalent to that of the fumigated laboratory. Therefore, further investigation applying the approach described in this paper could utilize real-time hydrogen peroxide sensors both inside the laboratory and BSC III that would allow for a direct comparison of hydrogen peroxide concentrations, enabling a better understanding of the decontamination profile and chemical stability throughout the cycle.

This method will provide end-users, technology developers, and regulatory agencies the opportunity to investigate the efficacy of fumigant decontamination technologies against various select agents while challenging them with all the complexities of room- or building-scale decontamination. Previous bench-scale decontamination studies have established benchmarks for efficacy of decontamination for many fumigation technologies; however, more studies are needed to generate larger-scale decontamination efficacy data. Building size, material complexity, as well as other factors, may directly impact the technology selected or the manner in which that technology is implemented to successfully conduct the decontamination. Confidence in the ability of fumigants to inactivate spores and other persistent threat agents requires the establishment of efficacy data for these targeted select agents in room- and building-scale environments.

Acknowledgments

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References


A Survey of Bioscience Research and Biosafety and Biosecurity Practices in Asia, Eastern Europe, Latin America, and the Middle East

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¹Sandia National Laboratories, Albuquerque, New Mexico, and ²BioInformatics, LLC, Arlington, Virginia

Abstract

In the past decade, the United States (U.S.) has enacted extensive federal legislation to regulate the possession, use, and transfer of dangerous microorganisms and toxins. Yet, few international laboratories have implemented similar safeguards. Limited data are available concerning the types of biological agents researched in non-U.S. laboratories and the biosafety and biosecurity practices employed to maintain those agents. To start addressing these knowledge gaps, an online survey was administered by BioInformatics, LLC in 2005 to 765 life scientists from 81 countries in Asia, Eastern Europe, Latin America, and the Middle East. Survey results revealed that participants are actively engaged in research with a wide variety of biological agents. Moreover, analysis of the biosafety and biosecurity data revealed several interesting findings; these findings are summarized into three major themes: biosafety is more prevalent than biosecurity, simple practices and techniques predominate, and perceptions of risk vary regionally. This survey provided unique insight into the variety of dangerous microorganisms and their toxins studied worldwide and uncovered a consistent weakness in laboratory biosafety and biosecurity. Because many of these facilities are located in volatile areas of the world, these findings indicate a potentially significant risk, and future actions are warranted to improve the safe and secure handling of biological agents internationally.

Introduction

Biosafety and biosecurity are fundamental practices that must be consistently implemented and enforced in any active bioscience research laboratory. As critical as these concepts are to the health and well-being of bioscience employees as well as the environment, an alarming lack of global awareness is associated with biosafety and biosecurity. Moreover, confusion in basic terminology exists as multiple definitions of biosafety and biosecurity are in circulation, and the terms are often used interchangeably. For example, biosafety often refers to the protection of the environment from genetically modified organisms; in animal industries, biosecurity refers to the protection of animals from microbial contamination. In regions that do recognize biosafety within a laboratory framework, biosecurity may not be independently recognized, or it is interpreted to mean the same as biosafety.

The World Health Organization (WHO) has been instrumental in promoting the necessity of laboratory safety since 1983, providing international guidance on basic biological safety and developing national codes of practice for the safe handling of pathogenic microorganisms in a laboratory setting. The WHO defines laboratory biosafety as “the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release” (WHO, 2004). In 2004, the WHO defined laboratory biosecurity as “institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens and toxins” (WHO, 2004). The American Biological Safety Association (ABSA, www.absa.org) and the European Biosafety Association (EBSA, www.ebsa.eu) are two other internationally recognized professional organizations that have been fundamental in establishing laboratory biosafety and biosecurity programs around the world, and they each define biosafety and biosecurity similar to these WHO definitions.

In the past decade, these and other organizations have contributed to a greater knowledge and commitment to laboratory biosafety and biosecurity in the global scientific community. Amerithrax (the case name referring to the 2001 Bacillus anthracis attacks in the U.S.) and other recent laboratory accidents and releases have alerted governments to the importance of biosafety and biosecurity, provoking the establishment of major economic and political initiatives to minimize the consequences of such biological risks. The United States, in particular, has been a global leader in promoting laboratory biosafety and biosecurity. The United States has enacted specific legislation to regulate the possession, use, and/or transfer of Select Agents, and violations carry criminal and civil penalties. Two key U.S. laws include the USA PATRIOT Act and the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. Many other countries have enacted similar national regulations.

Nevertheless, even with some of the most stringent safety and security safeguards in place, since 2003 American laboratories handling dangerous microorganisms and toxins have experienced more than 100 accidents involving such high-risk agents as Bacillus an-
**Articles**

thracicis, highly pathogenic avian influenza virus A (H5N1), monkeypox virus, and *Yersinia pestis*, and the number is increasing; biosafety infractions are also numerous (Kaiser, 2007; MSNBC, 2007; Ramshaw, 2007). While some information is available from the United States, very little is known about how non-U.S. laboratories are implementing biosafety and biosecurity. Also very little data are openly available that address biosafety or biosecurity incidents around the world, especially in developing countries.

To better assess the state of international laboratory biosafety and biosecurity, Sandia National Laboratories’ International Biological Threat Reduction (SNL IBTR) and BioInformatics, LLC administered an online survey to 765 scientists from 81 countries in Asia, Eastern Europe, Latin America, and the Middle East. Survey questions pertained to:

1. The level of awareness and understanding of biological risks in non-U.S. laboratories
2. The types of dangerous agents actively studied in non-U.S. laboratories
3. The types and practices of safety and security needed to maintain non-U.S. laboratories

Survey results revealed that a significant proportion of international researchers who study infectious agents practice poor biosafety and biosecurity. It is hoped that by identifying weaknesses through studies such as this, gaps in the development and implementation of biosafety and biosecurity measures can be addressed and, ultimately, overcome.

**Method**

**Survey Participants**

A cross-sectional online survey was administered to non-U.S. scientists, healthcare professionals, laboratory staff, and physicians between September 2005 and January 2007. Participants were recruited from two source populations maintained by BioInformatics, LLC. Over 47,108 scientists from Asia, Eastern Europe, Latin America, and the Middle East were invited to participate; 5,176 respondents completed the survey (response rate = 11.0%). Inclusion criteria required all participants to be actively engaged in bioscience research with at least one infectious agent and/or toxin. Respondents were excluded if they did not indicate research on an infectious agent and/or toxin, or completed less than approximately 85% of the survey.

The final analysis included an eligible sample of 765 participants from 81 countries. These individuals were categorized into two groups, Advanced and Emerging/Developing, based upon the state of their country’s bioscience research infrastructure. This infrastructure was determined using secondary research which considered scientific citations per country, life scientists per capita, government investment in life science research, and the presence of biotechnology parks. Countries with the highest number of responses per region are India (Asia; 102/300 or 34.0%), Poland (Eastern Europe; 22/146 or 15.1%), Mexico (Latin America; 35/165 or 21.0%), and Iran (Middle East; 41/154 or 26.6%). More than half the respondents were from the Advanced countries (58.8%) and were employed in an academic field (56.6%) as professors or teachers (32.2%). Basic research was the most frequently cited type of laboratory research conducted by participants from all regions (43.4%). Additional demographic information is summarized and provided in Table 1.

**Survey**

The online survey was developed to assess international biosafety and biosecurity practices. Each survey consisted of 30 questions, including 29 closed or partially closed questions and 1 open-ended question. Qualified scientists from each region were contacted by e-mail and instructed to access the questionnaire online. Survey questions focused on:

1. The types of dangerous pathogens and/or toxins used in research
2. Research objectives as they pertain to these pathogens and/or toxins
3. Laboratory capacity including tools, techniques, personnel, and physical structure
4. Status quo for biosafety and biosecurity
5. Perceptions of risk
6. Standards and accountability measures

The nearly universally recognized WHO Laboratory Biosafety Manual was used as the reference point for definitions of biosafety levels and other terms in an attempt to mitigate the diversity of terminologies which refer to different degrees of containment laboratories and different ways of implementing those controls.

The survey also collected information regarding participant demographics. Each survey was designed to take no more than 15 minutes to complete and guaranteed privacy to the respondent. Surveys were translated into English, Spanish, Russian, and Arabic. As encouragement for participation, participants were awarded redeemable points or a U.S. $10 honorarium.

**Survey Analysis**

Survey results in each region were tallied and analyzed by BioInformatics, LLC using a commercial software package, Statistical Package for Social Sciences (SPSS) (SPSS Inc., Chicago, Illinois). The response rate for each question varied as not all respondents answered the survey completely. All unanswered questions were treated as missing values in the analysis.

**Organization of This Paper**

Most of the respondents’ laboratories perform various types of research and work on multiple types of
### Table 1
Demographic factors of survey participants by response status (n [%]).

<table>
<thead>
<tr>
<th>Survey</th>
<th>Asia (n=300)</th>
<th>Eastern Europe (n=146)</th>
<th>Latin America (n=165)</th>
<th>Middle East (n=154)</th>
<th>Total (n=765)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL INVITED</td>
<td>17,786</td>
<td>10,211</td>
<td>8,128</td>
<td>10,983</td>
<td>47,108</td>
</tr>
<tr>
<td>Responded</td>
<td>1031 (5.8%)</td>
<td>1455 (14.2%)</td>
<td>879 (11.1%)</td>
<td>1811 (16.5%)</td>
<td>5176 (11.0%)</td>
</tr>
<tr>
<td>Eligible participants</td>
<td>300</td>
<td>146</td>
<td>165</td>
<td>154</td>
<td>765</td>
</tr>
<tr>
<td>Ineligible participants and/or incomplete surveys</td>
<td>731</td>
<td>1,309</td>
<td>714</td>
<td>1657</td>
<td>4,411</td>
</tr>
<tr>
<td><strong>Representative Countries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td>21</td>
<td>19</td>
<td>25</td>
<td>81</td>
</tr>
<tr>
<td>Advanced countries</td>
<td>6 (37.5%)</td>
<td>6 (28.6%)</td>
<td>7 (36.8%)</td>
<td>8 (32.0%)</td>
<td>27 (33.3%)</td>
</tr>
<tr>
<td>Emerging/developing countries</td>
<td>10 (62.5%)</td>
<td>15 (71.4%)</td>
<td>12 (63.2%)</td>
<td>17 (68.0%)</td>
<td>54 (66.7%)</td>
</tr>
<tr>
<td><strong>TOTAL RESPONSES</strong></td>
<td>300</td>
<td>146</td>
<td>165</td>
<td>154</td>
<td>765</td>
</tr>
<tr>
<td>Advanced countries</td>
<td>162 (54.0%)</td>
<td>73 (50%)</td>
<td>117 (70.9%)</td>
<td>98 (63.6%)</td>
<td>450 (58.8%)</td>
</tr>
<tr>
<td>Emerging/developing countries</td>
<td>138 (46.0%)</td>
<td>73 (50%)</td>
<td>48 (29.1%)</td>
<td>56 (36.4%)</td>
<td>315 (41.2%)</td>
</tr>
<tr>
<td><strong>Educational Level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TOTAL RESPONSES</td>
<td>279</td>
<td>145</td>
<td>164</td>
<td>152</td>
<td>740</td>
</tr>
<tr>
<td>Bachelors degree</td>
<td>13 (4.7%)</td>
<td>2 (1.4%)</td>
<td>10 (6.1%)</td>
<td>8 (5.3%)</td>
<td>33 (4.5%)</td>
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<tr>
<td>Masters degree</td>
<td>63 (22.6%)</td>
<td>20 (13.8%)</td>
<td>37 (22.6%)</td>
<td>12 (7.9%)</td>
<td>132 (17.8%)</td>
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<tr>
<td>Doctoral degree</td>
<td>200 (71.7%)</td>
<td>123 (84.8%)</td>
<td>116 (70.7%)</td>
<td>132 (86.8%)</td>
<td>571 (77.2%)</td>
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<tr>
<td><strong>Scientific Field</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TOTAL RESPONSES</td>
<td>277</td>
<td>145</td>
<td>164</td>
<td>154</td>
<td>740</td>
</tr>
<tr>
<td>Academic</td>
<td>138 (50.0%)</td>
<td>84 (57.9%)</td>
<td>96 (58.5%)</td>
<td>101 (65.6%)</td>
<td>419 (56.6%)</td>
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<tr>
<td>Government</td>
<td>46 (16.6%)</td>
<td>25 (17.2%)</td>
<td>36 (22.0%)</td>
<td>14 (9.1%)</td>
<td>121 (16.4%)</td>
</tr>
<tr>
<td>Hospital or medical center</td>
<td>34 (12.3%)</td>
<td>14 (9.7%)</td>
<td>12 (7.3%)</td>
<td>20 (13.0%)</td>
<td>80 (10.8%)</td>
</tr>
<tr>
<td>Pharmaceutical/biotechnology</td>
<td>28 (10.1%)</td>
<td>0</td>
<td>5 (3.0%)</td>
<td>3 (1.9%)</td>
<td>36 (4.9%)</td>
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<tr>
<td><strong>Employment Position</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TOTAL RESPONSES</td>
<td>282</td>
<td>146</td>
<td>164</td>
<td>154</td>
<td>746</td>
</tr>
<tr>
<td>Lab director/supervisor</td>
<td>46 (16.3%)</td>
<td>39 (26.7%)</td>
<td>39 (23.8%)</td>
<td>19 (12.3%)</td>
<td>143 (19.2%)</td>
</tr>
<tr>
<td>Professor/teacher</td>
<td>86 (30.5%)</td>
<td>37 (25.3%)</td>
<td>36 (22.0%)</td>
<td>81 (52.6%)</td>
<td>240 (32.2%)</td>
</tr>
<tr>
<td>Staff scientist</td>
<td>20 (7.1%)</td>
<td>24 (16.4%)</td>
<td>15 (9.1%)</td>
<td>13 (8.4%)</td>
<td>72 (9.7%)</td>
</tr>
<tr>
<td>Principal investigator</td>
<td>60 (21.3%)</td>
<td>22 (15.1%)</td>
<td>55 (33.5%)</td>
<td>15 (9.7%)</td>
<td>152 (20.4%)</td>
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<tr>
<td><strong>Type of Research Conducted in Laboratory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL RESPONSES</td>
<td>296</td>
<td>145</td>
<td>164</td>
<td>153</td>
<td>758</td>
</tr>
<tr>
<td>Basic</td>
<td>115 (38.9%)</td>
<td>73 (50.3%)</td>
<td>85 (51.8%)</td>
<td>56 (36.6%)</td>
<td>329 (43.4%)</td>
</tr>
<tr>
<td>Disease surveillance</td>
<td>54 (18.2%)</td>
<td>25 (17.2%)</td>
<td>41 (25%)</td>
<td>25 (16.3%)</td>
<td>145 (19.1%)</td>
</tr>
<tr>
<td>Clinical</td>
<td>56 (19.0%)</td>
<td>24 (16.6%)</td>
<td>22 (13.4%)</td>
<td>52 (34.0%)</td>
<td>154 (20.3%)</td>
</tr>
<tr>
<td>Drug discovery and/or development</td>
<td>57 (19.3%)</td>
<td>20 (13.8%)</td>
<td>9 (5.5%)</td>
<td>13 (8.5%)</td>
<td>99 (13.1%)</td>
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<tr>
<td><strong>Work Activities Using Infectious Agents and/or Toxins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL RESPONSES</td>
<td>276</td>
<td>143</td>
<td>162</td>
<td>152</td>
<td>733</td>
</tr>
<tr>
<td>Conduct “hands-on” research</td>
<td>108 (39.1%)</td>
<td>46 (32.2%)</td>
<td>70 (43.2%)</td>
<td>42 (27.6%)</td>
<td>266 (36.3%)</td>
</tr>
<tr>
<td>Supervise research</td>
<td>118 (42.8%)</td>
<td>58 (40.6%)</td>
<td>66 (40.7%)</td>
<td>74 (48.7%)</td>
<td>316 (43.1%)</td>
</tr>
<tr>
<td>Analyze data only</td>
<td>25 (9.1%)</td>
<td>25 (17.5%)</td>
<td>14 (8.6%)</td>
<td>22 (14.5%)</td>
<td>86 (11.7%)</td>
</tr>
</tbody>
</table>
pathogens; therefore, the participants’ survey responses are not research- or pathogen-specific. Rather, they reflect the cumulative or highest biosafety and biosecurity practices used throughout the laboratory. For example, a laboratory that primarily researches Salmonella at Biosafety Level 1 (BSL-1) might select Biosafety Level 3 (BSL-3) and other more stringent biosafety practices because a small proportion of its work is done with more dangerous pathogens. To better understand the research and type of pathogen-specific biosafety and biosecurity practices, the authors separated the respondents into two major categories based on their laboratory’s type of research and type of pathogen studied. This strategy removed all respondents who worked on more than one type of pathogen and in more than one research area.

Five research stages were considered: basic, clinical, disease surveillance, drug discovery, and translational research. Similarly, four types of pathogen research were considered: food-borne (Escherichia coli 0157:H7, Salmonella typhi, Shigella dysenteriae, and Vibrio cholerae), emerging (highly pathogenic avian influenza [A] virus A (H5N1), Hantaan virus, and severe acute respiratory syndrome coronavirus [SARS-CoV]), blood-borne (dengue fever viruses 1-4, human immunodeficiency virus type 1 [HIV-1], rabies virus, and Plasmodium sp.), and agents that pose an inhalational risk (SARS-CoV, Brucella abortus, melioidosis or suis, Chlamydo phila psittaci, Francisella tularensis, and Mycobacterium tuberculosis).

The survey consisted of simple yes/no or multiple choice questions to aid in data normalization. Tables 1-3 show the results of these analyses. The Biosecurity and Risk Perception portion of the survey uses a 5- and 7-point scale, respectively. The 5-point scale captures frequency of use (1= None of the time, 2= Little of the time, 3= Some of the time, 4= Most of the time, 5= All of the time), and the 7-point scale assesses severity of risk (1= Very Unconcerned/Very Unlikely, 7= Very Worried/Very Likely). Survey results are presented as a regional and overall average (Tables 4-5). This paper highlights and discusses only select biosafety and biosecurity practices and perceptions of risk in the sample population. The full results of the survey are published in SAND Report 2009-0868 available at: www.biosecurity.sandia.gov.

Results

Infectious Microorganisms and Toxins

Respondents represent such diverse fields as virology, microbiology, toxicology, and pathology and perform research on a wide variety of organisms. In general, respondents study bacteria and viruses more frequently than they study toxins. Bacteriology predominates in most areas; however, viruses are studied most often in Latin America. Regional variations in pathogen research presumably exist as a result of the epidemiological differences of endemic diseases and public health threats. Following are the most frequently studied agents and toxins common to each region.

The most commonly investigated pathogens and toxins overall are:

- *Escherichia coli* 0157:H7
- *Salmonella typhi*
- *Shigella dysenteriae*
- Human immunodeficiency virus (HIV)
- *Staphylococcus aureus* toxin

In all regions, *Escherichia coli* 0157:H7, *Salmonella typhi*, and *Shigella dysenteriae* are the three most frequently studied bacterial strains. *Mycobacterium tuberculosis* is a commonly researched pathogen in the Eastern European, Latin American, and the Middle Eastern regions, while *Vibrio cholerae* is the third most frequently studied infectious bacteria in Asia.

HIV is the most frequently studied virus in all regions. Other virus research varies widely. For instance, the large majority of Middle Eastern viral researchers study hepatitis viruses (70%) compared to just 14% of the Asian researchers. Yet Asia investigates SARS, highly pathogenic avian influenza, and dengue fever much more frequently than any other region.

Toxins and parasites were the least studied agents. Most respondents investigate *Staphylococcus aureus* toxins and malaria more than any other toxin or parasite. Botulinum toxin and *Clostridium perfringens* toxin were generally the second and third most researched toxins. In some Latin American regions, parasites such as *Trypanosoma cruzi*, the cause of the Chagas’ disease, are routinely studied. It is also important to note that up to 77% of researchers also reported working on biological agents and/or toxins not listed on the questionnaire. In these cases, respondents were able to select “Other” and list alternate agents researched in their laboratories.

Biosafety Practices

Personal protective equipment (PPE) is the most consistently used biosafety practice in all regions (Tables 2a-2b). Gloves, gowns, and lab coats are the most common PPE and are routinely worn 72%-94% of the time. Face shields and goggles are also common but used less frequently. Biosafety cabinets (BSC) and the presence of an autoclave within the laboratory are also fairly common in many of the respondents’ labs. More sophisticated biosafety practices, such as the ability to monitor people using closed circuit television and two-way communication, are the least employed practices. Other measures, such as building ventilation systems and effluent waste decontamination systems, are used sporadically in many regions. Detailed data were not collected on specific biosafety practices or on validation mechanisms of engineering controls.
Table 2a
Frequency of select biosafety practices (%).

<table>
<thead>
<tr>
<th></th>
<th>Monitor w/Window</th>
<th>Monitor w/CC TV</th>
<th>Monitor w/2-way Communication</th>
<th>BSC</th>
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A review of biosafety level practices revealed two significant findings. First, the majority of respondents work in a BSL-2 setting (Tables 3a-3b); this is consistent across all regions. However, some research is performed in BSL-1 and BSL-3 facilities. BSL-1 is used most frequently in food-borne pathogen research, while much of the emerging, blood-borne, and inhalational pathogen research is performed in a BSL-3. The most alarming finding, however, is that a significant proportion of the respondents claim they do not know at what biosafety level they currently work with their infectious agent or toxin. This was the most common response from the Middle Eastern participants.

Biosecurity Practices

Biosecurity is inconsistently employed around the world. Physical security to reduce the risk of unauthorized access to a laboratory is relatively weak in nearly every region. The most widespread biosecurity measures include locked laboratory doors, locked building doors,

Table 2b
Frequency of select biosafety practices (%).

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Tables 3a and 3b
The biosafety level most commonly used when working with infectious agents and/or toxins (%).

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and locked cabinets; less routinely researchers lock their refrigerators. Yet, in some areas, even these simple measures are not regularly implemented. More sophisticated physical security measures are used by a smaller proportion of respondents. These may include implementation of more technological measures or other advanced practices used to mitigate the risk of biological theft from a laboratory. In all categories, the use of video monitors, sensors and alarms, and self-closing doors are rarely used. Other practices vary extensively (Tables 4a-4b).

Personnel security is the principal measure for addressing the risk that a lab worker with legitimate access may steal or misuse a biological agent (Salerno & Gaudioso, 2007). For respondents, personnel security

### Table 4a
Physical Security Practices*

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*This question was answered on a 5-point scale: 5=All of the time; 4=Most of the time; 3=Some of the time; 2=Little of the time; 1=None of the time. The above scores are an average of the respondents’ results.*
most typically involves biosecurity training for new employees, restricted access to laboratory areas, and lists of employees who have access to restricted areas. Overall, personnel security measures are even less consistently applied than physical security measures. Photo identification badges, building escorts, biosecurity training, and background screening of potential new employees are infrequently implemented. Generally, basic and clinical labs have very poor personnel security, with more advanced laboratories implementing more security measures.

Information security, a set of tools and practices used to protect sensitive information, is no less varied. In all regions, password protection of computers and files is the most frequent information security measure. In contrast, storage of important hard copies of information (including paper, tapes, and videos) in secure containers is the least practiced measure within each region and category. Lastly, Material Control and Accountability (MC&A) measures help enhance laboratory biosecurity by establishing exactly what biological material is present at a facility, how and where the material is stored and handled, and who is responsible for it (Salerno & Gaudioioso, 2007). Overall, MC&A is generally quite robust within each region as nearly all of the practices are used most to all of the time. The laboratory head or direct supervisor was the most commonly identified person to account for a laboratory's pathogens and toxins.

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*This question was answered on a 5-point scale: 5=All of the time; 4=Most of the time; 3=Some of the time; 2=Little of the time; 1=None of the time. The above scores are an average of the respondents’ results.
Risk Perceptions

When asked about the respondent’s degree of concern for various scenarios involving infectious agents and/or toxins that his/her laboratory studies, the most commonly cited concerns for most categories included **Accidentally infecting people or animals or contaminating the environment outside the laboratory and Laboratory-acquired infections** (Table 5a). Yet, respondents researching emerging pathogens often selected **Becoming more virulent** and **Acquiring ability to infect new species** as highly likely (i.e., a 5.8 and 6.2 average, respectively, on a 7-point scale where 7=Very worried).

Overall, being **Repurposed by government to serve as a public health lab (e.g., diagnostics, vaccinations, etc.) during an epidemic** was a significant concern in all regions. This was especially a concern in Asian, Eastern European, and Latin American laboratories doing disease surveillance work (Table 6a). Yet, when asked about the likelihood that their laboratory could be involved in or affected by **Theft of samples with the intent to do harm** by either an employee or a non-employee (outsider), the overwhelming majority of respondents claimed this was a remote possibility (Table 6b). This was consistent among both categories and across all regions.

**Table 5a**

Risk perceptions of various scenarios involving an infectious agent or toxin that a respondent’s laboratory studies by research type.*

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<th>Acquire Ability to Infect New Species</th>
<th>Agent or Toxin Could Be Used to Cause Harm</th>
<th>Become More Virulent</th>
<th>Laboratory-acquired Infection</th>
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*This question was answered on a 7-point scale: 7=very worried; 1=very unconcerned. The above scores are an average of the respondents’ results.*
Discussion

This survey sought to gather insight into the status of international laboratory biosafety and biosecurity in Asia, Eastern Europe, Latin America, and the Middle East. The survey engaged 765 international life scientists who actively study infectious agents and/or toxins. Specific objectives of the survey include identifying the types of pathogens and/or toxins used in research, research objectives, laboratory capacity, the status quo for biosafety and biosecurity policies and procedures, perceptions of risk, and standards and accountability measures. Results of the analysis identified three major themes:

1. Biosafety is more prevalent than biosecurity.
2. Simple practices and techniques predominate.

Biosafety is More Prevalent than Biosecurity

Biosafety is implemented, to varying extents, in every region included in this survey. A primary reason respondents may employ biosafety is to reduce the risk of accidentally infecting themselves while working with infectious pathogens. This concern is likely the principal reason why personal biosafety—in the form of lab coats, gloves, and safety goggles—is relatively universal. Interestingly, although personal safety might be important to the respondents, environmental safety is not, as just one-third to one-half of respondents routinely decontaminate their waste, and roughly one-quarter to one-third use controlled ventilation. Evidence also suggests that for many, a known risk that cannot be mitigated will result in work not being conducted, as approximately 50%-80% of respondents claim that if they do not have a particular piece of laboratory safety equipment neces-

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*This question was answered on a 7-point scale: 7=very worried; 1=very unconcerned. The above scores are an average of the respondents’ results.
sary to perform an experiment, they will not perform the experiment. However, what a respondent deems as “necessary equipment” is most likely influenced by his/her prior laboratory experience. In addition, respondents’ organizations use a variety of different means to manage their biosafety and biosecurity programs. The most commonly used methods are a biosafety operations manual, an institutional biosafety committee, biosafety training procedures, and a laboratory management plan, although biosecurity programs are not frequently used. Biosecurity issues also do not figure as predominantly in most of the respondents’ risk assessments.

Nevertheless, other biosafety practices, which are necessary for specific types of research, are used less regularly. For example, dangerous biological agents that have the potential to be inhaled during experimental procedures require additional biosafety practices and measures, such as a biological safety cabinet (BSC), one of the most important pieces of laboratory equipment to reduce the risk of inhalational exposure. Unfortunately, BSCs are used by only 64% of researchers who study pathogens that pose an inhalational hazard. The survey did not examine whether BSCs are regularly, or annually, certified. In addition, many of the laboratories that work

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*This question was answered on a 7-point scale: 7=very likely; 1=very unlikely. The above scores are an average of the respondents’ results.
on some of the most dangerous pathogens do not commonly use controlled access measures (double-door entry, physical isolation of the laboratory), and, moreover, these laboratories do not appear equipped to handle accidents, as the majority of them lack a sealable room for decontamination or an anteroom. Enhanced biosafety measures such as a double-ended autoclave, an anteroom with a shower, and closed-circuit television are rare.

While many respondents appear to be concerned about the natural route of infection or an agent’s pathogenicity, the majority of respondents studying dangerous infectious agents do so at one biosafety level lower than that recommended in applicable guidance from the World Health Organization and/or the Centers for Disease Control and Prevention (CDC). In Asia, for example, nearly two-thirds of respondents investigating Japanese encephalitis, avian influenza, and SARS perform their research under BSL-2 conditions.

One explanation for this discrepancy is that some of these labs are diagnosing clinical specimens for which BSL-2 work practices would be appropriate. Another possibility is that respondents’ laboratories are not able—due to lack of resources—to work at the appropriate biosafety level. Alternatively, respondents may claim to be working at one biosafety level but are actually employing more stringent safety practices. In many cases, the respondents are not aware of their laboratory’s official biosafety level or are not entirely clear what the various biosafety levels are or when they are needed. For example, one respondent from Pakistan reported using a BSL-4 when researching food-borne pathogens such as *E. coli* and *S. typhi* in his/her laboratory. Another Pakistani reported using a BSL-4 for research in a basic research laboratory while a respondent from Jordan reported using a BSL-4 for research in a clinical laboratory. Yet, no evidence in the public literature confirms that either of these countries operates a BSL-4 facility. Moreover, further reviews of individual biosafety responses do not support the engineering controls consistent with a BSL-4 laboratory.

### Table 6b

Risk perceptions that a respondent’s laboratory would be involved in or affected by one of the following scenarios.*

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<td><strong>4.0</strong></td>
<td><strong>2.2</strong></td>
<td><strong>2.1</strong></td>
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*This question was answered on a 7-point scale: 7=very likely; 1=very unlikely. The above scores are an average of the respondents’ results.
In general, fewer laboratories utilize biosecurity-related measures than biosafety-related measures. An overwhelming majority consider the Theft of samples with the intent to do harm by an employee or non-employee highly unlikely. While most respondents’ laboratories employ minimal biosecurity measures, the fact that the awareness level and perceived threats about biological terrorism is so low suggests that these measures are likely not implemented to the standards recommended by the WHO. Many of the respondents who are not implementing biosecurity are engaged in research with dangerous endemic agents, such as foot-and-mouth disease virus, dengue fever virus, SARS-CoV, and avian influenza virus. In addition, many of the respondents who do not consider the Theft of samples with the intent to do harm by an employee or non-employee a concern also specifically identify a biosafety concern, Accidentally infecting people, animals or the environment.

Simple Practices and Techniques Predominate
Simple biosafety and biosecurity measures predominate in every survey category, while more sophisticated and expensive measures are used by a significantly smaller proportion of respondents who, in most cases, reside in wealthier countries. As previously mentioned, the most common biosafety practice is the use of personal protective equipment such as gloves, goggles, and gowns. Expensive technologies like two-way communication, positive pressure suits, and pass-through autoclaves are rarely used. Furthermore, most of the sophisticated techniques and equipment are located in the handful of BSL-4 laboratories scattered throughout the survey regions. When asked about the types of laboratory techniques used with infectious agents and/or toxins, the majority of respondents reported using simple tools routinely, for example classical polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISAs), and electrophoresis. Advanced genotyping and gene expression analysis technologies, such as single nucleotide polymorphism (SNP), RNA interference (RNAi), and deoxyribonucleic acid (DNA) microarrays, are used only by a small percentage of respondents.

Biosecurity shows a similar trend. Laboratories located in developing countries tend to choose more personnel-intensive security approaches, relying on guards as their main source of protection. Many of these laboratories simply post a guard at the building entrance and lock their cabinets, building, and laboratory doors; the use of engineered security controls such as intrusion sensors, alarms, and video monitors are rare in developing countries. Personnel-based security measures are inherently less effective; electronic systems are more reliable and have the advantage of providing constant monitoring.

Perceptions of Risk Vary Regionally
In general, the overall perception of risk varied among regions. Latin American and Asian respondents were the most concerned about various scenarios involving an infectious agent or toxin in their laboratories (Tables 5a and 5b). When considering the survey’s five types of laboratory risk, Latin Americans respondents were consistently most concerned with Accidentally infecting people, animals or the environment and Laboratory-acquired infections across all research type categories, except Latin American labs that perform disease surveillance and translational research. Latin American respondents who study drug discovery research were most concerned with each of the survey’s proposed laboratory risks; Asian disease surveillance researchers were also concerned with all of the risks (Table 5a).

Similarly, all Asian research laboratories were most concerned with Discovering an Emerging Infectious Disease (Table 6a). Being Repurposed by government to serve as a public health lab during an epidemic is a concern for Latin American labs researching emerging pathogens, blood-borne pathogens, and pathogens that present an inhalational hazard (Table 6b).

Generally, Middle Eastern respondents who study pathogens that present an inhalational hazard are the most worried about the laboratory risks presented in the survey, while Eastern European researchers of the same category were the least concerned about all the risks (Table 5b). Furthermore, regional analysis of biosafety levels reveals that Middle Eastern respondents consistently do not know at which BSL level they currently work (Table 3a).

It is important to note that when risk perceptions of individual countries in each region were analyzed, no trends could be identified. The responses of participants from various institutions within a region, even within the same country, differed dramatically. For example, in two Indian disease surveillance labs, one categorized the lab scenario risk as extremely high, while another found absolutely no risk. This was common in both wealthy and poor countries. The only discernable trend in the risk perception data was that respondents from the wealthier countries collectively perceive much greater risk than the poor countries, perhaps indicating better awareness.

Conclusions
This work has highlighted the strengths and weaknesses of laboratory biosafety and biosecurity internationally. Survey results revealed that participants are actively engaged in research with a wide variety of biological agents, including Salmonella typhi, Escherichia coli O157:H7, Shigella dysenteriae, HIV, and Staphylococcus aureus toxin. Mycobacterium tuberculosis is also commonly researched in many countries, while Asia investigates SARS, highly pathogenic avian influenza, and dengue fever much more frequently than any other region. In contrast, biosafety and biosecurity practices around the world are varied. Results of these analyses were summarized into three major trends or themes:
biosafety is more prevalent than biosecurity, simple practices and techniques predominate, and perceptions of risk vary regionally.

One reason why biosecurity is employed less often is because laboratory biosecurity is a relatively new concept, even in U.S. laboratories. In regions which do recognize biosafety within a laboratory framework, biosecurity may not be independently recognized, or it is interpreted to mean the same as biosafety. This susceptibility is likely to be even greater for those laboratories that strongly rely on security provided by humans (e.g., entry guards or security patrols) rather than technology, a trend that is more typical of laboratories located in Developing/Emerging countries than in Advanced countries.

Respondents cite a variety of reasons for poor research practices. In all regions, respondents claimed a lack of funding and other resources as the primary factor that prevents laboratories from applying appropriate biosafety and biosecurity measures. A lack of funds, especially in the Developing/Emerging countries, prohibits laboratories from purchasing the necessary equipment and hiring qualified staff. A lack of awareness or education is also a significant hurdle. Many respondents are guided by their employers or employee training, but biosafety training is minimally conducted in all regions. Furthermore, nearly every region reports looking to its country’s government to help shape its biosafety and biosecurity practices in the laboratory. Yet, for many countries, no national regulations or guidance exists.

A lack of a perception of risk also exists. Interestingly, when considering the individual countries that have been affected by SARS or avian influenza, several respondents reported their most significant worry was Laboratory-acquired infections and Increased virulent strains; however, the majority did not utilize the necessary biosafety precautions to mitigate those risks. It is likely that institutes that lack the appropriate biosafety practices will have frequent laboratory-acquired infections, although no information is available in the open source literature to confirm this. Even so, many respondents’ ignorance or dismissal of the possible harm a particular pathogen might cause, in the event of accidental exposure, is worrying.

The authors acknowledge the inherent limitations and shortcomings associated with this survey data, and, therefore, do not recommend that its results be used alone to sufficiently or confidently assess risk or to generate conclusions that could potentially shape policy. Most surveys, including this survey, are seldom truly random since responses are voluntary; participants may be more likely to respond if they feel strongly about a subject, potentially resulting in a bimodal distribution of data. Furthermore, the members of The Science Advisory Board represent a segment of the scientific community with a demonstrated willingness to participate in market research activities. These factors may inject a certain level of bias into the findings presented in this report.

The points or honoraria given to participants were designed to counterbalance this possibility, perhaps by motivating those less likely to be responsive. However, approaches such as these may result in a situation where financially poor institutions respond more frequently, thereby artificially overestimating regional risk and under-representing comparatively well-funded institutions that may have better risk-reducing practices and equipment in place. Moreover, certain groups or subgroups may simply be over-represented in the participant pool or be more likely to respond to a survey for cultural reasons. For example, Indian scientists responded most frequently to the Asia survey; thus, India is represented to a greater degree relative to other countries. Also apparent is that, for many survey questions, data stratification greatly reduced the sample size, thereby limiting the statistical power of the study. In addition, this survey targets just four regions of the world; therefore, generalizing these data to other regions of the world may or may not be appropriate. Similar surveys are planned in the future for Africa, North America, and Western Europe.

Lastly, although laboratory policies and procedures are critical for the successful implementation of biosafety and biosecurity, these are harder to measure objectively in a survey format; therefore, this survey focused predominately on whether laboratories had specific items in place to address biosafety and biosecurity (e.g., the existence of written documentation, locks on doors, badges, biosafety cabinets, etc.). This is a fundamental limitation of the way this survey was designed since many laboratories around the world successfully handle dangerous pathogens without staff acquiring infections by implementing strong procedural controls and training instead of relying on engineered controls. Therefore, this survey structure should not be interpreted as a recommendation for engineered controls over procedural controls.

Nonetheless, given a thorough consideration of the potential caveats associated with survey analyses, surveys are an important tool for collecting data necessary to make firmer, more informed risk assessments of broad, often intractable problems, and, therefore, the authors believe this survey provides many valuable insights. To date, surveys of biosafety and biosecurity practices are rare and relatively narrow in scope. This survey is exceptional in that it presents a summary of data from various types of countries in four different regions of the world.

In addition, these findings are supported by previously published incidents and evidence of negligent biosafety and biosecurity practices, domestically and internationally. In Europe, an onsite audit of 22 facilities, including 94 Danish laboratories, found poor biosecurity widely prevalent, with open access to freezers containing lethal viruses and bacteria, such as Bacillus anthracis, foot-and-mouth disease virus, and Yersinia pestis; 90%
of the laboratories studied did not conduct regular inventory checks or perform personnel background checks (Bork et al., 2007). Similarly, in 2006, Texas A&M was cited for a variety of biosecurity problems, ranging from unauthorized access to high-security labs, missing vials of infectious agents including Brucella bacteria, improper disposal of infected animals, negligence in using personal protective equipment, and failure to report exposure of laboratory workers, to dangerous biological agents (Kaiser, 2007; Ramshaw, 2007). Other recent high-profile biosafety breaches include the laboratory-acquired infections of SARS in 2003 and 2004, and the accidental release of foot-and-mouth disease virus from a laboratory in the United Kingdom in 2007 (Health and Safety Executive, 2007; Taylor et al., 2005).

This survey shows that much work remains to be done internationally to safely and securely manage infectious agents and toxins and to reduce the risk of exposure and theft. Establishing a culture of safety, security, and responsibility will be immensely challenging, especially in those areas of the world that do not perceive the risks and/or lack adequate resources to mitigate those risks. So that the United States can contribute more effectively to the ongoing efforts to promote biosafety and biosecurity, more information needs to be collected about laboratory policies and practices worldwide. When combined with insights obtained through complementary and collaborative relationships with partners in this field, this wealth of data can be used to better raise global awareness of the critical issues involved with developing, implementing, and overseeing biosafety and biosecurity measures in bioscience institutes around the world.

References


Uniting and Strengthening America by Providing Appropriate Tools Required to Intercept and Obstruct Terrorism (USA PATRIOT Act), Public Law 107-54 October 26, 2001. Available at: https://www.biosecurity.sandia.gov/legislation


The Transfederal Taskforce on Biosafety and Biocontainment Oversight Report

The purpose of the Task Force is to propose options and recommendations to improve biosafety and biocontainment oversight of research and research-related activities at high and maximum containment laboratories in the United States, without hindering the progress of science. The scope of activities considered by the Task Force includes those that occur in all high and maximum containment research laboratories in all sectors (government [Federal, State, Tribal, and municipal], academia, privately funded research institutions, and private industry) utilizing potentially hazardous biological agents. The activities covered include research with disease-causing agents (pathogens) that can infect humans, zoonotic agents that can infect both animals and humans, biologic toxins, and agricultural pathogens and pests. Also included are activities related to research, such as the maintenance of facilities and equipment needed for effective biosafety and biocontainment, incident-reporting, and public outreach and communication efforts. Available at: www.hhs.gov/aspr/omsph/biosafetytaskforce/index.html
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.

Laboratory Doors—Open or Closed?

The following question is paraphrased from a recent post to the ABSA List Serve. I thought it deserved attention because it is a common problem in all laboratories, not just the biocontainment laboratories. Many facilities have laboratories that have doors to public hallways that are kept access-controlled. Within these areas, there are doors to multiple individual lab rooms and shared equipment rooms in which multiple responsible users may be working on a variety of Risk Group 1 and Risk Group 2 projects. Lab personnel are resistant to keeping doors closed because of the inconvenience of moving back and forth to the shared spaces.

Question
Does lab staff need to keep the doors closed? Leaving doors to laboratories in the open position seems to be a universal problem in laboratory facilities, whether they are for biocontainment or other types of research. Researchers and other laboratory personnel feel inconvenienced if they have to open doors to move from one area to another. So why should doors be kept closed?

Answer
First, working in a laboratory can be inherently dangerous. Whenever hazardous chemicals, radioactive materials, or biological hazards are being used, a chance of spill and potential exposure exists. In addition, in many laboratories personnel are working with potentially flammable material and there is the chance of a fire, which can spread to other areas of the building.

Second, it is an established fact that laboratories should be at negative pressure to the surrounding public areas in order to protect non-laboratory personnel from potential exposure to the airborne hazards of the laboratory. The air flow should be from public areas and offices to the laboratories and from areas of lower potential contamination to areas of higher potential contamination within the laboratories. This directional air flow is attained by increasing the amount of exhaust air that is removed from the laboratory to a level higher than that which is supplied to the laboratory. The make-up air then, ostensibly, comes from the corridor or public areas and creates a barrier that keeps contaminants inside the laboratory.

The easiest and most efficient way to provide directional air flow while keeping exhaust air to a minimum is to balance the ventilation system while the doors to the laboratory are closed. If these doors are closed, then the area through which the air flows into the laboratory is at a minimum and the air velocity is increased creating a pressure differential resulting in directional air flow into the lab. If, however, the door is left open, a large opening is created, the pressure differential drops, and the directional airflow, while still present, becomes negligible. Under these conditions the directional airflow is easily overcome by various perturbations and eddy currents in the laboratory and potential contamination can be released to the corridor or adjacent laboratory space. Open doors are also a particular problem when there is a fire in the laboratory since the heat from the fire can cause smoke to escape into other parts of the building.

Third, while it is possible to provide sufficient directional air flow through open doors, a significant increase in exhaust air would be required. Since the possibility for potential contamination of laboratory air with hazardous materials is substantial, the laboratory ventilation system is generally designed to be single-pass air that is not recirculated to the rest of the building. With the current concern for minimizing energy consumption, any increase in exhausting conditioned air without recouping the energy used to condition that air is not acceptable. The high exhaust air flow would also make it nearly impossible to open or close doors, depending on the swing of the door, and substantial noise would be associated with the excessive air flow. In addition, the increased air flow would cause drafts and perturbations of the air in the laboratory, which would be perceived by the occu-
pants as uncomfortable and could result in increased potential exposure to any hazardous materials being used in the laboratory.

So, should the doors be closed? Yes, the building is designed to work with the doors closed. Are the closed doors really an inconvenience for those working in the laboratory? They may think they are, but considering the alternatives, keeping the doors closed is a small price to pay. Red lights at intersections are an inconvenience to me when I’ve failed to correctly plan how long it will take me to get somewhere, but they are a necessity and I’ve learned to put up with them. The researchers in the laboratory should understand the need for keeping the doors closed and learn to close them for their own safety and for the safety of others in the building.

Question
How do you deal with the situation where a number of different researchers share open laboratories and different hazards are present? Should you provide information about all hazards including biologicals, chemicals, and radioactive materials that are present anywhere in the lab to all personnel in the lab?

Answer
Yes. In an open lab, a spill anywhere in the lab could affect anyone in the laboratory space and all personnel working in the space must be informed of the potential hazards even if they are not working directly with them. Personnel should also be aware of signs and symptoms of exposure to the hazardous materials and be trained to handle emergency situations should they arise.

Capsule

Ed Krisiunas

WNWN International, Burlington, Connecticut

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

Interim Guidance on Infection Control Measures for 2009 H1N1 Influenza in Healthcare Settings, including Protection of Healthcare Personnel

CDC is releasing updated interim guidance on infection control measures to prevent the transmission of 2009 H1N1 influenza in healthcare facilities. The updated guidance applies uniquely to the special circumstances of the current 2009 H1N1 pandemic and will be updated as necessary as new information becomes available throughout the course of this influenza season. Revisions from earlier guidance include: criteria for identification of suspected influenza patients; recommended time away from work for healthcare personnel; changes to isolation precautions based on tasks and anticipated exposures; expansion of information on the hierarchy of controls that ranks preventive interventions in the following order of preference—elimination of exposures, engineering controls, administrative controls, and personal protective equipment; and changes to guidance on the use of respiratory protection. Available at: www.cdc.gov/h1n1flu/guidance/ill-hcp.htm

Summary of Notifiable Diseases—United States, 2007

Published July 9, 2009, Volume 56, Number 53

The Summary of Notifiable Diseases—United States, 2007 contains the official statistics, in tabular and graphic form, for the reported occurrences of nationally notifiable infectious diseases in the United States in 2007. Unless otherwise noted, the data are final totals for 2007 reported as of June 30, 2008. These statistics are collected and compiled from reports sent by state health departments and territories to the National Notifiable Diseases Surveillance System (NNDSS), which is operated by CDC in collaboration with the Council of State and Territorial Epidemiologists (CSTE). This Summary also includes publications from previous years. Available at: www.cdc.gov/mmwr/summary.html

Novel Influenza A (H1N1) Virus Infections Among Healthcare Personnel—United States, April-May 2009

MMWR, 58(23), June 19, 2009, pp. 637-660

Soon after identification of novel influenza A (H1N1) virus infections in the United States in mid-April 2009, CDC provided interim recommendations to reduce the
risk of transmission in healthcare settings. These included recommendations on the use of personal protective equipment (PPE), management of healthcare personnel (HCP) after unprotected exposures, and instruction to ill HCP not to report to work. To better understand the risk for acquiring infection with the virus among HCP and the impact of infection-control recommendations, CDC solicited reports of infected HCP from state health departments. As of May 13, CDC had received 48 reports of confirmed or probable infections with novel influenza A (H1N1) virus; of these, 26 reports included detailed case reports with information regarding risk factors that might have led to infection. Of the 26 cases, 13 (50%) HCP were deemed to have acquired the infection in a healthcare setting, including one instance of probable HCP to HCP transmission and 12 instances of probable or possible patient to HCP transmission. Eleven HCP had probable or possible acquisition in the community, and two had no reported exposures in either healthcare or community settings. Among 11 HCP with probable or possible patient to HCP transmission and available information on PPE use, only three reported always using either a surgical mask or an N95 respirator. These findings suggest that transmission of novel influenza A (H1N1) virus to HCP is occurring in both healthcare and community settings and that additional messages aimed at reinforcing current infection-control recommendations are needed. Available at: www.cdc.gov/mmwr/preview/mmwrhtml/mm5823a2.htm

Potential Impact of a Two-Person Security Rule on Biosafety Level 4 Laboratory Workers

Emerging Infectious Diseases, 15(7), July 2009 (online report) available at: www.cdc.gov/eid/content/15/7/e1.htm

Directors of all major Biosafety Level 4 (BSL-4) laboratories in the United States met in 2008 to review the current status of biocontainment laboratory operations and to discuss the potential impact of a proposed two-person security rule on maximum-containment laboratory operations. Special attention was paid to the value and risks that would result from a requirement that two persons be physically present in the laboratory at all times. A consensus emerged indicating that a video monitoring system represents a more efficient and economical standard, provides greater assurance that pathogens are properly manipulated, and offers an increased margin of employee safety and institutional security. The two-person security rule (one to work and one to observe) may decrease compliance with the dual responsibilities of safety and security by placing undue pressure on the person being observed to quickly finish the work and by placing the observer in the containment environment unnecessarily.


Calendar of Events

February 28—March 5, 2010
ABSA Principles & Practices of Biosafety
Embassy Suites, Ft. Lauderdale, Florida
Contact:  Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

March 15-18, 2010
ABSA Review Course and Spring Seminar Series
Sheraton, New Orleans, Louisiana
Contact:  Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

September 30—October 6, 2010
ABSA 53rd Annual Biological Safety Conference
Hyatt Regency Denver at Colorado Convention Center, Denver, Colorado
Contact:  Phone: 1-866-425-1385 or 847-949-1517; Fax: 847-566-4580; E-mail: absa@absa.org; www.absa.org

October 10-14, 2010
American Association for Laboratory Animal Science (AALAS) 61st National Meeting
Atlanta, Georgia
Contact:  http://nationalmeeting.aalas.org/future_sites.asp
The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at mlambert@mcw.edu or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Recombination and Horizontal Gene Transfer: Routes to Increased Bacterial Pathogenicity

A previous Molecular Biosafety column (Lambert, 2008) laid out the basic premise for why the potential for genetic recombination should be considered in risk assessments of pathogens: because recombination represents a key route to change in nucleic acids. The current article continues this series with a focus on recombination in bacterial pathogens.

Bacterial Recombination

Introduction of new genetic material/ altered sequences into bacteria can be through several mechanisms including:

- Mutations: Some mutational mechanisms involve recombination, resulting in rearrangements such as deletions, duplications, or insertions.
- DNA Repair: Most DNA repair mechanisms involve recombination at some level. For bacteria, a main route of repair is homologous recombinational repair which has recombination as its centerpiece.
- Horizontal Gene Transfer (HGT): The introduction of extrachromosomal mobile units plays a central role in bacteria gaining genetic material. HGT allows bacteria to respond quickly to changing environments and to exploit new ecological niches (Sobecky & Coombs, 2009). HGT is largely responsible for the spread of fitness-enhancing traits, including antibiotic resistance and virulence factors (Averhoff, 2009).

In bacteria, genetic material is often introduced via HGT with mobile genetic elements such as plasmids (conjugation) or bacteriophages (transduction) and through the uptake of naked DNA that is integrated into the bacterial chromosome (natural transformation) (Kelly et al., 2009). Though recombination is not involved in HGT itself, DNA that has been introduced via HGT can then be recombined with other mobile genetic elements or with the bacterial chromosome. The bacterial environment is so dynamic that new advantageous genetic material can be maintained on mobile genetic elements indefinitely through selective pressure. However, genetic material that benefits the bacterial host is also maintained through integration into the bacterial chromosome (through recombination mechanisms including transposition “jumping genes”). Integration enables vertical gene transfer (VGT) when the bacteria replicates.

Thus, recombination plays a major role in the introduction of genetic material to bacteria, which is counterbalanced by HGT. Likewise, recombination plays a major role in the maintenance of gene expression through integration into the bacterial genome, but this role for recombination is also counterbalanced by selective pressure that results in the maintenance of new genetic material that is beneficial to that specific bacteria in that particular environment.

Risk Assessment Considerations for Recombination in Bacterial Pathogens

Risk assessments of bacterial pathogens need to take into account whether introduced changes can result in increased virulence, increased pathogenicity, broadened host or tissue ranges, increased routes of transmission, decreases in infectious dose, increased survival in the environment, or increased resistance to antibiotics. HGT is often facilitated by genomic islands (GEIs), discrete DNA segments which can be mobile. Many GEIs can integrate into the host chromosome, be readily excised, and transfer to a new host by transformation, conjugation, or transduction. GEIs involved in processes affecting pathogenicity, such as rapid dissemination of genes coding for antibiotic resistance genes and for virulence genes, are termed pathogenicity islands (Juhas et al., 2009). The rapid evolution of pathogenicity islands involves both HGT and recombination mechanisms.

Type IV secretion systems are conjugation-related genes that are very versatile. They can be involved in protein secretion and in the export and import of novel DNA sequences that are then integrated into the bacterial genome (natural transformation). Thus, type IV secretion systems are tools for HGT and for increasing virulence (Juhas et al., 2008).

Biofilms are microbial communities that are matrix-embedded and consist of interacting microorganisms. The organisms within a biofilm communicate by gene transfer and by secretion of signaling molecules. Gene transfer mechanisms in biofilms often involve HGT, but may also include recombination mechanisms. Biofilm formation in pathogens often results in increased virulence and decreased susceptibility to antimicrobial
agents (Marsh, 2005).

One review article (Ambur et al., 2009) evaluates genome dynamics in a number of major bacterial pathogens with a focus on key mechanisms (DNA repair, recombination, and HGT) that enable maintenance of bacterial genome integrity. DNA repair mechanisms (with recombination generally having a fundamental role in the mechanism) contribute to the ability of bacteria to colonize, transmit, and survive inside the host. While the absence of DNA repair activities may be beneficial and allow adaptation during some stages of a pathogen’s life cycle, intact repair systems are essential for long-term colonization. The Ambur review article concludes that these divergent needs regarding DNA repair can be met through bacterial pathogens having mechanisms such as HGT for speedily reacquiring genes encoding DNA repair functions.

The relative importance of recombination as the mechanism of change is species-specific and sometimes even strain-specific and is also dependent on the adaptive response in the host of that pathogen. Streptococcus pyogenes, for example, demonstrates high levels of recombination and a narrow habitat (Bessen, 2009) while Streptococcus pneumoniae has a high level of HGT by natural transformation (Johnsberg & Havarstein, 2009). Mycobacterium tuberculosis shows significant genetic diversity generated by recombination mechanisms, but unlike many bacterial pathogens, gene exchange is rare resulting in distinct clonal lineages in M. tuberculosis (Nicol & Wilkinson, 2008). Population studies indicate that recombinational mechanisms play a large role in speedy acquisition of antibiotic resistance in commensal species of Neisseria meningitidis (Sáez-Nieto & Vazquez, 1997). Putative virulence factors of Helicobacter pylori are numerous and are often acquired through recombination events involving pathogenicity islands (Figura, 1997). In Staphylococcus aureus, antibiotic resistance genes are often exchanged between organisms via HGT, but chromosomal mutation (which can be via a recombination mechanism) is the catalyst for novel resistance genes (Jensen & Lyon, 2009).

In summary, both HGT and recombination are important mechanisms in acquisition of new traits in bacterial pathogens, and both selective pressures and recombination are important routes to maintain those traits beneficial to that organism in that environment. HGT and recombination mechanisms, however, are often not separable events, but instead are complementing mechanisms that allow bacteria, including pathogenic bacteria, to quickly adapt to changing environments (Sriramulu, 2008).

Doing risk assessments that take into account the various mechanisms that can result in increased bacterial pathogenicity is very complex. It is clear, however, that getting as complete a picture as possible for specific pathogens enables a more thorough risk assess-ment and allows tailoring of precautions based on the specific risks posed by that particular pathogen.

**Author’s Note**

See upcoming Molecular Biosafety columns in this journal for further discussion on evaluating potential recombination risks for various types of pathogens.

**References**


Book Review

Reviewed by Jean E. Feagin

University of Washington, Seattle, Washington

The Elusive Malaria Vaccine: Miracle or Mirage?

By Irwin W. Sherman


Seeking Success—The Search for an Effective Malaria Vaccine

Malaria kills a child every 30 seconds. The World Health Organization currently estimates 247 million cases of malaria annually, with just under a million dead each year. Most of those are children under the age of 5, living in sub-Saharan Africa. Beyond the enormous health burden, major economic effects are also evident, reflecting in large part the loss of productive capacity due to illness. Malaria is estimated to have reduced the Gross Domestic Product of endemic countries by up to 50% over the last 30 years, compared to non-endemic countries.

Today, malaria is largely confined to tropical and subtropical areas of the world, but as recently as 150 years ago it was also found across much of Europe, large swaths of Asia, throughout the eastern United States, and along the Pacific coast as far north as Vancouver Island. Any disease of such import attracts many people who want to cure or prevent it, be they motivated by a desire to do good or to win glory. Toward the end of the 19th century, malaria research was hot: the bloodstream parasite was identified by Laveran in 1880 and the mosquito stages and transmission by Ross in 1897 and 1898, respectively. Vaccinology was also making important strides, including successful vaccines using weakened and killed pathogens. The two fields were synergistic. With the pathogenic agent for malaria in hand, the search for a vaccine was on. Today, over a century later, the search continues.

In The Elusive Malaria Vaccine: Miracle or Mirage?, Irwin Sherman recounts the pursuit of a malaria vaccine over the last century, writing from the perspective of ~50 years studying the biochemistry of malaria parasites. The book is both a history and a compendium of information about malaria vaccine research, supplemented by basics in parasite biology, immunology, and vaccinology. Key discoveries are described, along with the scientific milieu in which they were made. Approximately half the book discusses contemporary vaccine studies, including the pros, cons, and principal antigens for vaccine strategies that target different stages of the malaria parasite life cycle. Sherman also includes the sort of lore that begins as common knowledge, but then fades into obscurity: the idiosyncrasies of bird, rodent, and monkey malarias; immunological observations made during malaria therapy for syphilis.... For those who enjoy such tidbits, the book is a delight.

The book is engagingly written and should be of great interest not just to malaria researchers but also to anyone interested in the history of infectious diseases and interventions. (For those unfamiliar with malaria terminology, it may be useful to keep a thumb in the excellent index so that details can be checked as needed.) The historical aspect of the book is especially well done. Sherman reports key findings in malaria research and allied fields with more detail than usual, not just providing more information about key figures but also naming those whose contributions are often overlooked. Biographical information for well-known figures of the past—Pasteur, Koch, and Ross, to name just a few—is spiced with stories that convey the personalities and foibles of these giants. In another twist from the usual, similar information is included for contemporary scientists as well. Overall, Sherman chronicles the mysteries and answers, the hopes and disappointments, and the passions, bitter rivalries, and occasional scandals that have accompanied the search for an effective malaria vaccine.

There are a few distractions. Some sentences, typically long, complex ones, may need a second read to grasp the point. The recitation of results from early vaccination studies with animal models suffers from dryness and lacks a summation of what was learned from these experiments. And figures to illustrate the relationships of immune system components and to show the location of candidate vaccine antigens on the parasite would be helpful for readers unfamiliar with these. But these are small issues.

It is especially fitting to review the history of malaria vaccine research at this time. RTS,S, the most successful malaria vaccine candidate to date, has just entered a Phase III trial in 11 African countries. Although its effectiveness is only ~30%-50% (depending on the metric tested), it may reduce malaria deaths substantially. Initial trials are imminent for vaccines consisting of live attenuated parasites, delivered by mosquito bite. In the meantime, numerous labs around the world continue to search for the elusive malaria vaccine. It has been trumpeted many times that a malaria vaccine is imminent, with hopes dashed afterward. Major strides in malaria research during the last decade, coupled with increased visibility and funding for malaria vaccine research in the last decade, have raised hopes again. But is it enough? Sherman speculates in his final chapter that an effective malaria vaccine will take another 20-30 years. I can only hope that his prediction will prove to have been pessimistic.
This is an exciting opportunity for an Industrial Hygienist/Safety Manager to work at the U.S. Department of Homeland Security Plum Island Animal Disease Center (PIADC). PIADC is the Nation’s number one laboratory responsible for protecting livestock from foreign animal diseases. Scientists from both DHS and USDA work at this facility - located off the coast of Orient Point, NY, with access from both Orient Point and Old Saybrook, CT, by government ferry - where they conduct basic and applied research to better understand foreign animal diseases, develop vaccines, and maintain and improve the Nation’s foreign animal disease diagnostic capabilities.

We are seeking an energetic individual with superb leadership, managerial, technical, communication, and inter-personal skills with experience in planning, coordinating, implementing, and improving the biological safety programs, occupational health, industrial hygiene, and emergency response in support of the PIADC mission.

With a staff of federal and contracted technical experts, the individual selected will provide leadership and management for all safety and health services required to support PIADC’s mission. Major organizational duties include:

- Develop and improve policies, procedures and technical guidance.
- Develop and conduct training.
- Establish, implement, and improve performance management and reporting processes.
- Collaborate on plans and schedules for repairs, maintenance, renovations, and new construction.
- Develop and coordinate budget proposals to fund program requirements.
- Develop, monitor and oversee execution of contracts.
- Plan, develop and provide public presentations.
- Coordinate and liaise with regulatory authorities.

Candidates must be U.S. citizens and hold a degree in industrial hygiene or a branch of engineering, physical science or life science, or a combination of education and experience. Additional education requirements are outlined on the website identified below.

The selected applicant must successfully complete security background checks, including for Select Agents, and will be subject to random and applicant drug testing.

To apply to the vacancy announcement and see a copy of the qualifications, please go to http://www.usajobs.gov and search on announcement number ST-20100000. Applicants must address and respond specifically to the Knowledge, Skills and Abilities ("KSAs") criteria to be evaluated and considered for the position.

This position has a salary range of $106,776 - $138,815 per annum (GS-0690-14). Relocation costs may be paid. DHS is an Equal Opportunity Employer.
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Speakers include:
- Caryl Griffin, MSN, MDIV, Co-founder, Elizabeth R. Griffin Research Foundation
- Gary Fujimoto, MD, Physician/Consultant, Palo Alto Medical Foundation
- L. Casey Chosewood, MD, Senior Medical Officer, NIOSH, CDC
- Jonathan Holder, DO, MPH, Occupational Health Physician, Cambridge Health Alliance
- Elizabeth Gilman Duane, MS, CBSP, Associate Director, R&D Environmental Health & Safety, Pfizer Research
- LouAnn Burnett, MS, CBSP. Biosafety Program Manager, Vanderbilt University
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About the sponsors:

Eagleson Institute is a non-profit foundation with a mission to promote the principles and practices of laboratory safety.

American Biological Safety Association is a professional association that provides a forum for the exchange of biosafety information.

Elizabeth R. Griffin Research Foundation promotes safe research practices and helps fund research into zoonotic diseases. The Foundation was formed in memory of Beth Griffin, a researcher whose young life was prematurely ended after contracting macaque-born B virus (previously known as Herpes B Virus).

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