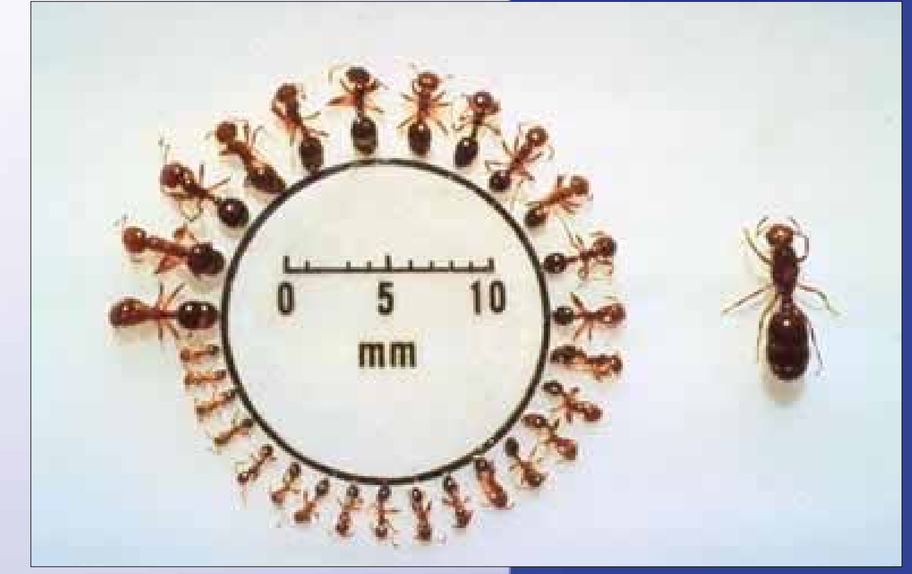
Applied Biosafety

Journal of the American Biological Safety Association



Volume 11, Number 2, 2006

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Applied Biosafety

Journal of the American Biological Safety Association

Volume 11, Number 2, 2006

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About the Cover

Imported fire ant workers (*Solenopsis spp.*) are arrayed to illustrate the size range of individuals. The large ant to the right is a reproductive queen (S. D. Porter, U.S. Department of Agriculture). Imported fire ants are aggressive, effective competitors of resources, economically costly, and have been described as posing serious health threats to plants, animals, and potentially humans. For laboratory and research related safety and containment information on fire ants, see "Safety Considerations for Handling Imported Fire Ants (*Solenopsis spp.*) in the Laboratory and Field" by James T. Vogt, et al., on pages 88-97.

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Mission Statement

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

Goals

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

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Glenn A. Funk

Gualala, California

Almost three years ago, our association, together with EBSA and colleagues from Det Norske Veritas (DNV), a Norwegian company specializing in risk management, recognized the potential value of an internationally-acceptable standard for laboratory biosafety and biosecurity based on a management system approach. ABSA, EBSA, and DNV proceeded to investigate the feasibility of this idea and we found that the European Commission was very interested in seeing such a standard developed. The EC was so interested, in fact, they indicated their willingness to contribute significantly to the cost of such an effort. DNV, ABSA, EBSA, and, ultimately, the Asia-Pacific Biosafety Association (A-PBA) developed a strong business plan based on many discussions and meetings. With the strong support of the ABSA Council, we have participated as a Team member for the past two years. Drs. Paul Huntley (DNV) and Stefan Wagener (ABSA) have presented updates on the progress of the effort at the past two annual ABSA Conferences and on other platforms, and our Past-President Betsy Gilman Duane wrote about this effort in her President's Page columns in *Applied Biosafety* last year. However, since the effort is now approaching reality, I want to, once again, tell all ABSA members about this important global project.

The vision of the project is to safeguard life, property, and the environment from biological risks through the development and adoption of internationally-recognized standards in the area of the management of biological materials, organisms and their products, primarily within the laboratory environment. The project encompasses several goals:

- Improve performance through the adoption of recognized good practice;
- Facilitate international exchange and collaboration;
- Increase awareness and adoption of management system approaches within the sector;
- Provide organizations with a means for internal audit and third-party certification; and
- Provide stakeholders with a standard to be used as a benchmark in setting requirements for facilities.

The aim of the project will be to develop an internationally-recognized standard and accompanying guidance document based on the WHO *Laboratory Biosafety Manual*. The main focus will be on biological agents and materials in a laboratory setting. The use of a management system approach (i.e., OHSAS 18001 or ISO 17025) will allow for a holistic approach encompassing people, facilities, and working procedures. It is our intent that the standard itself not be prescriptive in nature, but rather based on the philosophy that the operator must understand and manage the risks. Supporting the standard, the guidance document will be written to help organizations determine how adequate measures can be identified and implemented to satisfy the requirements of the standard. The combination of regulatory and guidance documents is commonly employed by European health and safety directorates; an American equivalent is, for example, the Bloodborne Pathogens Standard and the OSHA Compliance Directive used to guide field inspectors in enforcing the Standard. It's important to note that the proposed standard is, by design, voluntary and not intended to replace any national or sub-national regulatory requirements that may apply to the laboratory or facility.

We have chosen to develop this standard using the CEN Workshop model. CEN (Comité Européen de Normalisation, or the European Committee for Standardization, at www.cenorm.be) is an internationally-recognized standards development body, much like ISO. The CEN model brings stakeholders together at workshop sessions in which the stakeholders actually write the standard; stakeholders include institutions and companies with a vested interest in biosafety, and biosafety professionals from around the world. Consequently, it is not ABSA, EBSA, A-PBA, or DNV that write this standard. It is all of us as part of the international biosafety community with the aforementioned organizations primarily facilitating the process.

We envision developing working documents so that the first workshop is productive and not merely a training session for workshop participants. We believe that three or four workshops will ultimately yield a standard and guidance document accepted by participants and issued by CEN as what's called a CEN Workshop Agreement. The EC is familiar with the CEN model and recognizes DNV as a European company with strong experience in managing standards development under the CEN process.

It's very important that ABSA members recognize that they and their international biosafety partners bear the primary responsibility for writing this standard and developing the methods ultimately to be used to certify laboratories against the standard, and even to identify and set training requirements for the certifiers them-

selves. As mentioned before, the role of the DNV/ABSA/EBSA/APBA Team is to facilitate this process, with DNV overseeing the process as an experienced and acceptable manager of EC and other funds. The Team is being led by Stefan Wagener; I am a member of and an ABSA representative to this Team.

The current status of the project is as follows:

- A gap analysis of the WHO *Laboratory Biosafety Manual* has been completed, identifying those parts of the manual that can be used for a management system-based standard and those parts better suited for the guidance document;
- A budget, timeline and proposal has been submitted to the European Commission (EC); and
- A formal request for funding has been submitted to the EC on behalf of the four partners.

We are currently awaiting a decision by the EC; the Commission has indicated a willingness to provide as much as 75% of the approximately €500,000 budget; the remainder will come from workshop participants and non-European stakeholder sources. The ABSA Council has unanimously approved up to $10,000 in support of this effort and has confirmed its continuing commitment to the project. We expect the EC's decision in late summer and will have one year from the date of funding to complete the process.

Starting this summer, we will need to provide several very capable biosafety professionals to help write the working documents which will serve as the strawmen, or starting points, for the CEN Workshop process. Team members need to be ABSA members and must have a significant experience in biosafety and biosecurity and/or a solid working knowledge in quality management systems. Many of you have already told me of your interest in participating in this effort. Now is the time for you to express or confirm that interest by sending me a brief e-mail (biosafety@mcn.org) that includes your contact information.

By providing guidance and realistic design and performance criteria in a management systems context, this international standard promises to help propel biosafety into the 21st century and to extend its influence to those areas of the world where assistance is most needed.

CDC Meeting Summary

Submitted by Elizabeth Gilman Duane, Wyeth, Cambridge, MA

On March 7-8, 2006, several members of ABSA were invited participants at a Select Agent Biosecurity meeting convened by the Centers for Disease Control and Prevention (CDC) and facilitated by the Association of Public Health Laboratories. The goal of the meeting was to seek input from participants on how to provide more information on Select Agent Security Plan compliance for registered facilities and those applying for registration. The meeting, which was held in Atlanta, began with presentations covering Risk Assessment, Chain of Custody, Select Agent Security in Public Health and Academia, and Incident Response. The remainder of the meeting was dedicated to four focus groups that were tasked with evaluating certain aspects of the Security Section of the Select Agent regulations as well as draft guidance documents. Given that the Select Agent regulation is primarily a performance standard many registrants have had questions related to compliance. The 4 focus groups included: Risk Assessment, Risk Mitigation, Administration and Recordkeeping, and Guidance Documents. At the conclusion of the meeting each focus group reported on their findings and discussions. A publication of the meeting findings is slated to be completed 60 days from the conclusion of the meeting. The American Biological Safety Association appreciated the opportunity to participate.

EPA Pesticide Program Updates from EPA's Office of Pesticide Programs—3/28/06

New Telephone Line Established for Information about Antimicrobial Pesticides

The National Pesticide Information Center (NPIC) is now taking inquiries, via their telephone help-line and web-based services, regarding antimicrobial pesticides and pesticide products. Therefore, EPA's Antimicrobial Division hotline has been terminated, and EPA web pages with references to the Agency hotline are being updated to reflect this change.

NPIC is a toll-free telephone service that provides objective, science-based information about a wide variety of pesticide-related subjects. The service is available daily, 6:30 a.m. - 4:30 p.m. (PT), and the toll-free phone number is 1-800-858-7378.

Some Bioterrorism Issues of Quantitative Biosafety

Alexander Sabelnikov, Vladimir Zhukov, and Ruth Kempf

Center for Security Studies and Research, East Carolina University, Greenville, North Carolina

Abstract

With the increased recognition of a bioterrorism threat, the realm of biosafety is substantially expanding and merging with biosecurity. Two issues pertinent to bioterrorism and biosecurity, or biosafety in its new broader sense, are discussed in this article. The first concerns airborne exposure limits (AEL) for biological threat agents (including biowarfare (BW) agents) and a possible approach to determine these limits in the absence of precise data on infectious doses for most of these agents. The second issue concerns the sensitivity limits for the real-time biosensors that should detect BW agents and the significance of these limits for biosafety and biosecurity. To what levels of infection would the different sensitivity limits correspond and how is sensitivity related to the ability to manage the consequence of an airborne threat? These issues were addressed recently in a model study on detection limits of real-time biosensors (Sabelnikov et al., 2005) but because of their significance for biosafety and biosecurity deserve a much wider scope of discussion.

Introduction

Like most of the sciences dealing with biological entities, biosafety is rapidly changing both conceptually and methodologically. Not long ago its main concern was primarily laboratory safety. And though it is still considered "an inexact science" by some (e.g., one of the editors of *Biological Safety,* 2000 [Fleming & Hunt, 2000]), research in biosafety and related topics is making it a more exact and defined science with quantitative and prediction power. Examples include enhancements and refinements to areas such as risk assessment and analysis, sampling methods to detect contaminants, and novel methods developed over the past decade for decontamination. At present, with the advent of a bioterrorism threat, the realm of biosafety is substantially expanding and merging with biosecurity, even though according to the thesaurus, they are synonyms.

Never in the past was there a more significant threat concerning the use of BW agents by nonstate actors such as terrorists as the one that exists in modern times. Emergency response strategies (guidelines) to deal with any kind of terrorist event should incorporate several important factors such as immediate mitigation (medical, decontamination, etc.), organizational (sequestering the area, establishing a quarantine, evacuating the population, etc.), and postevent (clean up, decontamination, etc.) procedures. These should be based on scientifically proven exposure/health risk assessment for the materials involved (chemical, biological, or nuclear/radiological); effective detection, identification, and decontamination technologies; and accepted clean-up standards. In the case of a radiological terrorist event, when "dirty bomb" radiation could be detected and measured, the emergency response strategy has already been determined (NCRP, 2001). In the case of a terrorist event with the use of chemical warfare (CW) agents, the situation is not that clear because airborne exposure limits (AELs) obtained by extrapolation of toxicological data among animal species and from animals to humans have proven to be unreliable for many chemical agents (Johnson, 2003). For BW agents the situation is the worst: Lack of scientific data on infectious doses for most of them (Johnson, 2003; Raber et al., 2001) prevents the generation of their AELs. Furthermore, as recently concluded by the American Biosafety Association (ABSA), the attempts to develop quantitative values for human infectious dose are not currently feasible, and infectious dose values developed using past studies would not accurately characterize the relative hazard of pathogenic organisms in humans (Johnson, 2003).

Does this mean that AEL for bioterrorist agents cannot be generated at present and we have to wait years to fill the gap in our knowledge of infectious doses? The answer is "No." An efficient, temporary solution to bridge this gap and to set provisional AELs for BW agents may be inferred from the recent model study of detection limits of real-time biosensors—present, future, or conceivable (Sabelnikov et al., 2005). Several other issues addressed in this article also may be relevant to biosafety and biosecurity. For instance, recent Department of Homeland Security (DHS) agent sensitivity requirements for future field devices capable of BW agent identification in aerosols indicated a rather wide range of airborne concentration from 100 to 100,000 organisms per liter in air (DHS/HSARPA, 2004). Are these concentrations too high or too low with regard to biosafety and biosecurity? To what degree of "risk of infection" would these sensor sensitivity limits correspond? Will real-time biosensors be able to detect provisionary AEL for BW agents?

All these issues, because of their significance for biosecurity, or biosafety in its new, broader sense, deserve wider discussion among biosafety professionals.

Probability of Detection and Probability of Infection

Highly sophisticated and powerful biosensors, in addition to their use as constant surveillance devices and as mandatory equipment for the first responders, may also become working tools for laboratory biosafety personnel.

The evaluation of how the probability of detection corresponds to the probability of infection by an agent on the same time scale was achieved by a generated mathematical model. The model was based on the probabilistic nature of the events of detection of aerosolized microbes (including BW agents) and the events of infection, if they are pathogenic. The model included three generated equations for detection, one equation for the probability of infection by inhalation, and several general and specific assumptions. In particular, it was assumed that:

1. There is a space that contains aerosol particles with microbes. Microbes within aerosol particles and the latter within the space volume (both are *discrete* entities) are distributed according to Poisson distributions with parameters equal to their mean concentrations.
2. Aerosols contain both viable and nonviable (inactivated) forms of microbes. Only viable organisms are infectious; however, both forms can be identified.
3. Every *viable* pathogenic organism inhaled by the body (as aerosol) can initiate the infection process with a probability of p independently of other organisms.

Probability of Infection

The equation for the probability of infection by inhalation (equation 3 below) was derived based on the above assumptions from the earlier equations of Peto (1953), Mayorov et al. (1989), and Chermashentsev et al. (1993). In short, the probability that none of the ***viable*** organisms, N, infects the cell is $(1-p)^N$ can be approximated by $\exp(-pN)$. Further, according to the second assumption, $N = D_i D_v$, where D_i is a total number of inhaled viable and nonviable microorganisms, and D_v is the fraction of viable organisms. So, the probability of infection, **P,** may be written as:

$$P = 1 - \exp(-pD_iD_v) \quad (1)$$

By definition, **P** is equal to 0.5 when a dose becomes equal to **ID50** value (the infectious dose, or the number of microbes capable of producing the disease in 50% of those exposed). From that p can be easily found as:

$$p = \ln 2/ID50 \quad (2)$$

Substitution of p in equation (1) with its value from (2) yields the final equation for **P** that includes several key parameters, such as D_i, D_v, and **ID50**:

$$P = 1 - \exp(-D_iD_v \ln 2 / ID50 \quad (3)$$

Recently, a conceptual modeling approach was successfully used for the theoretical estimation of the risks of infection with smallpox virus and *Francisella tularenis* (Jones et al., 2005; Nicas et al., 2004). Though for most BW agents the infectious doses have not yet been precisely established (Johnson, 2003; Raber et al., 2001), there are reasons to believe that some agents, such as smallpox (Henderson et al., 1999; Nicas et al., 2004), tularemia (Dennis et al., 2001; Jones et al., 2005), and Q-fever (Fournier et al., 1998; Johnson, 2003), have a very low ID50 index (in single units). For others, like Marburg virus (Chermashentsev et al., 1993) and plague (Iglesby et al., 2000), it seems to be higher—approximately 100 units and approximately 1,000 units for anthrax (Iglesby et al., 2002; Meselson, 1994).

Taking these values into account, it seemed reasonable to group BW agents by their currently inferred ID50 values into three categories, with low, intermediate, and high ID50 values. It was suggested that such grouping might be an efficient, temporary solution for the purpose of the modeling (Sabelnikov et al., 2005). Apparently, it might also be efficient in setting "provisional" safety limits for BW agents. Three levels were assigned for inhaled ID50 indices and used for simulations: low (5 organisms), intermediate (100 organisms), and high (1,000 organisms).

Our first objective here is to consider what this approach and grouping provides with regard to provisional airborne exposure limits (AEL).

Similar to PEL (permissible exposure limits) for hazardous chemicals (including chemical warfare agents), several AEL may be considered for BW and other infectious agents depending on the *time of exposure* via the inhalation route. Further, AEL as a "permissible airborne concentration" of an agent would determine a certain, *critical risk of infection* by inhalation in a certain period of time (exposition). So, in order to find "safe," permissible, concentration limits, we first have to agree about what are the critical ("acceptable") risks of infection after a certain time of exposure for every BWA agent, and then find permissible AEL corresponding to those risks.

Taking into account that $D_i = t\, W_h\, C$ (where C is the concentration of an agent in the air, t is the time of exposure, and W_h is the adult inhalation rate), and making a simple rearrangement of equation 3, we obtain:

$$C = -\ln(1 - P)\, ID50 / (D_v\, t\, W_h \ln 2) \quad (4)$$

where all the symbols are the same as in equation (2 and 3). (Note that C is inversely dependent on D_v.)

In Table 1 airborne concentrations corresponding to different risks of infection after 1 hour exposure are presented for all three groups of BW agents. Throughout all the simulations that were performed with equation 4, 11 liters/min was used as the adult inhalation rate, W_h (Allan & Richardson, 1998).

Table 1

Airborne concentrations (microbes/m^3) corresponding to risks of infection by different ID_{50} groups of BW agents after 1 hour exposure.

Risk of Infection with BW agents (after 1hour)	Corresponding Airborne concentration of a BWA (units/cubic meter)			
	Bacillus anthracis (ID_{50}=1000)		Marburg virus (ID_{50}=100), Dv= 0.1	smallpox virus (ID_{50}=5), Dv = 0.1
	Dv= 0.5	Dv = 0.1		
0.000001	0.004358	0.021789	0.002179	0.000109
0.00001	0.043579	0.217894	0.021789	0.001089
0.0001	0.435808	2.179041	0.217904	0.010895
0.001	4.360045	21.80022	2.180022	0.109001
0.01	43.798	218.99	21.899	1.09495
0.1	459.1468	2295.734	229.5734	11.47867

Let us consider what risks of infection, if any, might be considered critical. Taking into account the lack of epidemiological potential of *B. anthracis*, even the value of about $1x10^{-4}$ for the risk of infection (column 1) might initially appear to be an acceptable candidate for a safety limit for this BW agent. That would correspond to AEL of 0.44 organisms/m^3 air (with 50% viability, Dv=0.5, column 2), and 2.18 organisms/m^3 air (with 10% viability, Dv=0.1).

The situation involving agents with epidemiological potential, such as smallpox, is, however, less clear. Zero risk might be a necessity in this case because according to the model and other recent theoretical estimations (Nicas et al., 2004), even tiny airborne concentrations of the agent seem to have unacceptable risks of infection. For instance, concentrations as small as 11 virions/1000m^3 still pose an infection risk of the order of $1x10^{-4}$ (Table 1). The final assessment in setting the critical risk level of infection and critical air concentrations for every particular BW agent should belong to all interested stakeholders, such as safety and occupational health professionals, medical doctors, emergency response authorities, regulatory agencies, public organizations, and the general public via regulatory review practices.

The results presented in Table 1 show that even very small airborne concentrations of BW agents, and especially highly infectious agents such as smallpox, are still able to provide significant risks of infection for adult humans. It is conceivable that such small airborne concentrations of BW agents might originate from a terrorist act when very small amounts of a BW agent are used, especially if the attack is conducted indoors. Tiny concentrations of a BW agent may also arise from the reaerosolation of BW agent remnants left after incomplete decontamination of the sites of terrorist attacks. The likelihood of such reaerosolation, especially for spore-forming agents, was suggested in the notorious case of *B. anthracis* accidental release from the warfare facility in Sverdlovsk in 1979 (Alibek, 1999) and demonstrated recently during the decontamination of affected indoor areas after the anthrax attacks of 2001 in the United States (Altman, 2001).

It is important to know whether these small concentrations of BW agents can be detected by real-time biosensors. This question was among the original goals of our earlier model study (Sabelnikov et al., 2005). The biosafety implications are discussed below.

Probability of Detection of BW Agents with Real-time Biosensors

In order to simultaneously estimate the probability of infection and the probability of detection of BW agents, the time of sampling (by a sensor) and the time of inhalation of infectious agent by an individual were set as equal. That allowed us to exclude from all calculations, if needed, time and concentration factors. The model yielded quantitative results upon the input of several incoming parameters such as an infectious dose of a microbe, parameters of a model sensor, etc. A model biosensor was defined as a single device that included an aerosol sampler and a device for identification by any known or conceived method. A network of biosensors was defined as a set of several single biosensors that operate in a similar way and deal with the same amount of agent. The three model biosensors used for the simulation employ different and widely used techniques (for a recent review, see Graham & Sabelnikov, 2004) to include polymerase chain reaction (PCR), antibody/antigen binding, and mass spectroscopy (MS). Neither the particular deployment of sensors within the network nor the spatial and

temporal distribution of agent aerosols due to wind, ventilation, humidity, temperature, etc. was considered by the model.

According to one of the assumptions of the model, the probability of agent identification in the individual sample was set equal to the probability of finding the agent in that sample in quantities not less than the threshold value, **I**, called the *sensitivity* of the biosensor. Further, it was assumed that the number of organisms in the sample volume V_s follows a Poisson distribution with a parameter, λ, equal to the mean concentration (# per volume) of viable and nonviable organisms in the sample volume. So, the probability of identification, P_{is}, of the agent in one sample with the volume V_s was expressed as:

$$P_{is} = 1 - \sum_{k=0,I-1} F(k), \quad (5)$$

where $F(k) = (\lambda V_s)^k e^{-\lambda V_s}/k!$ is the probability of finding exactly **k** organisms in the sample volume V_s , $\lambda = K_e D_i W_f/(W_h V_c)$—mean concentration of organisms, both viable and nonviable in collective, V_c , and individual, V_s , samples; a sampler intakes the air with a flow rate, W_s, and concentrates aerosolized particles with an efficiency, K_e, into a liquid collective sample of volume V_c .

Since a sensor may detect BW agents in several parallel samples, **n,** the probability of agent identification with one device, P_{id}, is equal to the probability of identification at least in one of **n** and was expressed as:

$$P_{id} = 1 - (1-P_{is})^n \quad (6)$$

By analogy, the probability of agent identification with the net of **m** similar devices, P_{im}, was expressed as:

$$P_{im} = 1 - (1-P_{id})^m \quad (7).$$

To simultaneously estimate the probability of identification and infection for various BW agents, several hundred simulations were performed for all three types of model sensors (see above) with variable parameters that encompassed metric features used in modern, commercially available biosensors, in laboratory devices, and only theoretically achievable. It turned out that an overwhelming amount of simulation data yielded extremely low values of probability of detection for doses less than 500 microbes, so only the simulation results obtained with the best existing or conceivable metric characteristics for a particular model sensor were presented in the paper (Sabelnikov et al., 2005). Evidently, those metric parameters provided the most beneficial conditions for detection, rarely achievable in real life with equipment currently used in the field or in laboratories. However, as was shown by the results of the simulations, none of the model sensors analyzed could identify the quantities of agents corresponding to inhalation doses equal to or less than D_i = 5 microbes.

Using the model we can compare how efficient the best model sensors are in detecting the infection risks presented in Table 1. Listed are some metrics of the model sensors that were used in the simulations. A model, PCR-based sensor with the *best combined* metrics of some currently available commercial devices (Idaho Tech. Inc., 2004; Smiths Detection, 2004; BDS/Invitrogen, 2004; Cepheid, 2004; Sceptor Industries, 2004; etc.) would be able to analyze 16 identical samples of an agent (**n**=16) of volume, V_s = 12.5 μl/sample, with the sensitivity, **I**, of 15 organisms. It would be attached to a sampler with W_s = 1000 l/min and K_e = 0.8. An antibody/antigen-based model sensor (such as that used by, for example, Laricchia-Robbio & Revoltella [2004], Naimushin et al. [2002], and Uttenthaler et al. [2001]) would combine the best available metrics: W_s=1000; K_e =0.8; V_c=10; V_s =1; **n**=4, and **I** = 25. The operational concept of MS-based model biosensor (such as that used by Doroshenko et al. [2002], Madonna et al. [2003], and Warscheid & Fenselau [2003]) employs the following metrics: Aliquots of 0.001 ml (V_s) would be withdrawn from 10 ml of concentrated collected sample (V_c) and applied onto a target plate. They would be evaporated, ionized with a laser beam, and analyzed. Accordingly, **n** is set as 1, and **I** as 1. All other parameter values are the same as for the other sensors (see above).

Detection of BW Agents with Real-time Model Biosensors

Table 2 shows the simulation results on detection of different doses of a BW agent with ID50 index of 1000 (*Bacillus anthracis, etc)* by the model PCR-, Antibody/antigen- , and MS-based sensors with the metrics described above.

Amazingly, both the model PCR-based and MS-based sensors failed to detect microbes of *B. anthracis*-level of infectivity (ID_{50}=1000) in doses 100 times higher than those providing suggested "safe"/accepted risk limits (0.0001). In fact, that dose could not be detected by any model sensor tested (Sabelnikov et al., 2005). A similar or even worse situation with regard to detection is observed with the more infectious agents (ID_{50} =100), such as Marburg virus, *Y. pestis*, etc. (Table 3), or smallpox virus (Table 4). In fact, those small, undetected doses can still provide rather high risks of infection even for the high level ID_{50} organisms, such as *B. anthracis*. In the case of aerosols containing highly viable microbes (with D_v > 0.1) of intermediate and high levels of infectivity/virulence (intermediate and low ID_{50} level organisms) and especially of those with high contagious potential, such as smallpox, lack of detection would have catastrophic epidemiological consequences.

As predicted by the model for PCR-, antibody-, and MS-based sensors, the reliable identification of small BW agent doses equal to or less than single microbes could be theoretically achieved either by increasing the identifier sensitivity or enhancing concentration of the agent in the sample. However, in reality it seems unlikely because the increase in sensitivity will undoubtedly result in raising

Table 2

Detection of different doses of a BW agent with ID_{50} index of 1000 by the model PCR-, Antibody/antigen-, and MS-based sensors with the best metric characteristics.

Risk of Infection	Dose of inhaled BW agent (ID_{50}= 1000)	Probability of detection, P_{id} of BW agents by:		
		PCR-based model biosensor	Antibody/antigen-based sensor	MS-based model biosensor
0.000001	0.002886	0	0	2.1E-05
0.00001	0.02886	0	0	0.00021
0.0001	0.288615	0	1.39E-13	0.002097
0.001	2.887447	0	0.615705	0.020781
0.01	29.0053	0	1	0.190184
0.1	304.0708	1	1	0.890455

Table 3

Detection of different doses of a BW agent with ID_{50} index of 100 by the model PCR-, Antibody/antigen-, and MS-based sensors with the best metric characteristics.

Risk of Infection	Dose of inhaled BW agent (ID_{50} = 100)	Probability of detection, P_{id} of BW agent by:		
		PCR-based model biosensor	Antibody/antigen-based sensor	MS-based model biosensor
0.000001	0.001443	0.00	0.00	0.00
0.00001	0.01443	0.00	0.00	0.00
0.0001	0.144307	0.00	0.00	0.00
0.001	1.443723	0.00	0.00	0.01
0.01	14.50265	0.00	1.00	0.10
0.1	152.0354	1.00	1.00	0.67

false positives, especially for highly "loaded" environmental samples. It is likely that the same factors will affect the increase of the agent concentration in the sample.

Overall, the results of simulations performed with variable metric parameters of all three types of model sensors (Sabelnikov et al., 2005) indicated that small doses of aerosolized agents (less than 5 microbes) that are still able to provide significant risks of infection especially for highly infectious agents (e.g., for smallpox those risk are 1, 8, and 37 infected out of 1,000 exposed, depending on the viability of the virus preparation) would remain undetected by the present, most advanced, or even future, significantly refined real-time biosensors. Meanwhile, they are expected to help in selecting the proper emergency response management (ERM) should a bioterrorist attack occur. The solution might be in using multicomponent networks of sensors (with more than 4 similar devices). However, it may be costly, at least at the present time.

Extending the Approach and the Results Beyond BW Agents

In contrast to the military strategy of BW agents' application, the main desirable effect of a bioterrorist attack is to cause massive panic and to paralyze economic and social activities. To achieve this it is not absolutely necessary to use deadly BW agents. Perhaps, it might be suffi-

cient to cause the outbreak of diarrhea or some other readily detectable disturbance among a good portion of civil population. So, other infectious agents such as those, for instance, that cause food poisoning (*Bacillus cereus, Salmonella* strains, etc.) might also be employed by bioterrorists. Interestingly, as mentioned in the Russian guidelines on countermeasures against biological terrorism (CABT, 2003), "aerosol distribution of microbes might be considered universal, because even microbes with non-inhalation routes of infection may be distributed in this way." Nothing is known, however, about their possible inhalation infectious doses, though intuitively we may assume that their "acceptable" safety limits should be orders of magnitude higher then those of BW agents. If they are indeed retaining their infectious potential as aerosols, the approach discussed here may be used also for determination of their AEL.

Conclusion

Two additional points need to be made. First, it should be emphasized that real-time identification, no matter how rapid and sensitive, cannot prevent infection. In the best cases it can mitigate the consequences and help with a proper emergency management. Additionally, most of the time it should be expected that real-time identification, if it indeed takes place, will estimate at best only the lowest possible doses inhaled by the exposed individual, since this individual keeps on breathing during the identification time. Therefore, the fastest identification will be extremely beneficial. In this respect, MS-based sensors capable of identification within minutes might be the most promising.

Second, AELs undetected by the model real-time biosensors closely correspond to, or even extend, the high and very high levels of Index of Microbial Air contamination, IMA, introduced by Pasquarella et al. (2000) for environments at risk. They also correspond to, or extend, the highest classes of air contamination defined by several authoritative agencies such as NASA (1967), U.S. Federal Standards 209E (1992), European Union Good Manufacturing Practices (1997), and International Organization for Standardization, ISO/DIS (1998). The environments at high risk usually include aseptic and operating rooms at hospitals, microelectronics and pharmaceutical plants, and others. High risks of infection by small doses of BW agents, especially of those with high epidemiological potential, add a new "member" to the class of environments at very high risk.

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Vaporized Hydrogen Peroxide-based Biodecontamination of a High-Containment Laboratory Under Negative Pressure

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Abstract

The authors evaluated vaporized hydrogen peroxide as an alternative to formaldehyde for space biodecontamination in a containment level 3 laboratory suite. The laboratory air pressure during the biodecontamination process was maintained at a slightly negative pressure. This was done as a preventive measure to ensure that hazardous vaporized hydrogen peroxide would not escape during the process. Parameters such as temperature, relative humidity, vaporized hydrogen peroxide concentration, and pressure within the laboratory suite were monitored during the biodecontamination. The success of the decontamination process was validated using spores of G. stearothermophilus, the most resistant microorganism to vaporized hydrogen peroxide (Kokubo et al., 1998; Meszaros, 2005; Rickloff & Orelski, 1989). This research demonstrates the usefulness of vaporized hydrogen peroxide as a space biodecontaminant.

Introduction

The Canadian Science Centre for Human and Animal Health houses 16 animal cubicles, five containment level 3 (CL3) suites, and seven CL4 suites, each containing individual laboratories. Since its opening in 1997, formaldehyde gas has been used to decontaminate the high-containment laboratories, animal cubicles, and biosafety cabinets. This process has often been slow, disruptive, and difficult to standardize (Krause et al., 2001; Spiner & Hoffmann, 1971). Additionally, formaldehyde gas upon neutralization polymerizes to paraformaldehyde and settles on the surfaces, warranting thorough postdecontamination clean-up. Porous materials such as wood, paper, and clothing absorb, retain, and release formaldehyde gas over time (Braswell et al., 1970). In addition to being a health hazard (Cogliano et al., 2004; *Lancet*, 1983; Rutala, 1990, 1996), a mixture of formaldehyde gas or paraformaldehyde dust in air has the potential to explode (WHO, 1994a; WHO, 1994b). Therefore, the authors have been exploring safer and automated alternative technologies for space decontamination.

Vaporized hydrogen peroxide (VHP)-based biodecontamination technology was developed in the 1980s and commercialized in the early 1990s (Graham & Rickloff, 1992; Heckert et al., 1997a; Rickloff & Graham, 1989). This technology has since been gaining popularity, now used for the decontamination of clean rooms, animal rooms, ambulances, large volume filling rooms, and hospital wards contaminated with antibiotic-resistant bacteria (French et al., 2004; Jahnke & Gerhard, 1997; Krause et al., 2001; Malmborg, 2001; Mitchell, 2005). VHP is known to be a powerful oxidizer and it inactivates viruses, fungi, bacteria, bacterial spores, nematode eggs, and even prions (Fichet et al., 2004; Heckert et al., 1997b; Kokubo et al., 1998; Krause & Riedesel, 2004; Meszaros, 2005). The VHP process is rapid, dry, mobile, compatible with electronics, and effective at low concentrations and temperatures. Unlike formaldehyde, VHP produces nontoxic by-products (water and oxygen) and, therefore, is ecologically safer and requires no postprocess neutralization and cleaning. However, a VHP concentration of over 75ppm is considered an immediate risk to human health; the accepted personal exposure level is under 1ppm (American Industrial Hygiene Association, 1957; National Institute for Occupational Safety and Health, 1996). In this study the authors have evaluated VHP biodecontamination in one CL3 laboratory suite. To address the safety concern, they maintained the laboratory suite at a slightly negative pressure to prevent VHP from escaping to the neighboring areas. Biological and chemical indicators were placed within the lab to assess the success of the VHP decontamination processes.

Materials and Methods

Laboratory Suite

The laboratory suite was built as a CL3 lab and has adjacent dirty change, shower-out, and clean change rooms. It had a volume of 3,000 cubic feet and contained biosafety cabinets (Class II Type A2 & Class III), incubators, refrigerator, freezer, centrifuge, telephone, fax ma-

chine, computer, security camera, microscope, and other routine laboratory equipment. The laboratory was supplied with conditioned air and exhausted through double HEPA filters directly to the outside.

Preparation for Biocontainment

For thorough circulation of VHP within the labspace, six oscillating fans were positioned inside the laboratory suite (Figure 1). Their locations and directions of oscillation were determined by a smoke test using Drager air current tubes. Additionally, the Class II BSC was left running to further enhance VHP distribution. To decontaminate the dirty change and shower-out rooms, the doors between the laboratory and dirty change room, and between the dirty change and shower-out rooms were held open. All the electronics and laboratory equipment were left *in-situ* to determine their compatibility to VHP. The VHP generator (STERIS® VHP 1000ED) was situated in the mechanical space above the laboratory. VHP was piped-in and the return air piped-out of the lab using two 1-1/2-inch stainless steel pipe penetrations in the concrete floor slab.

Biocontainment Program Cycle

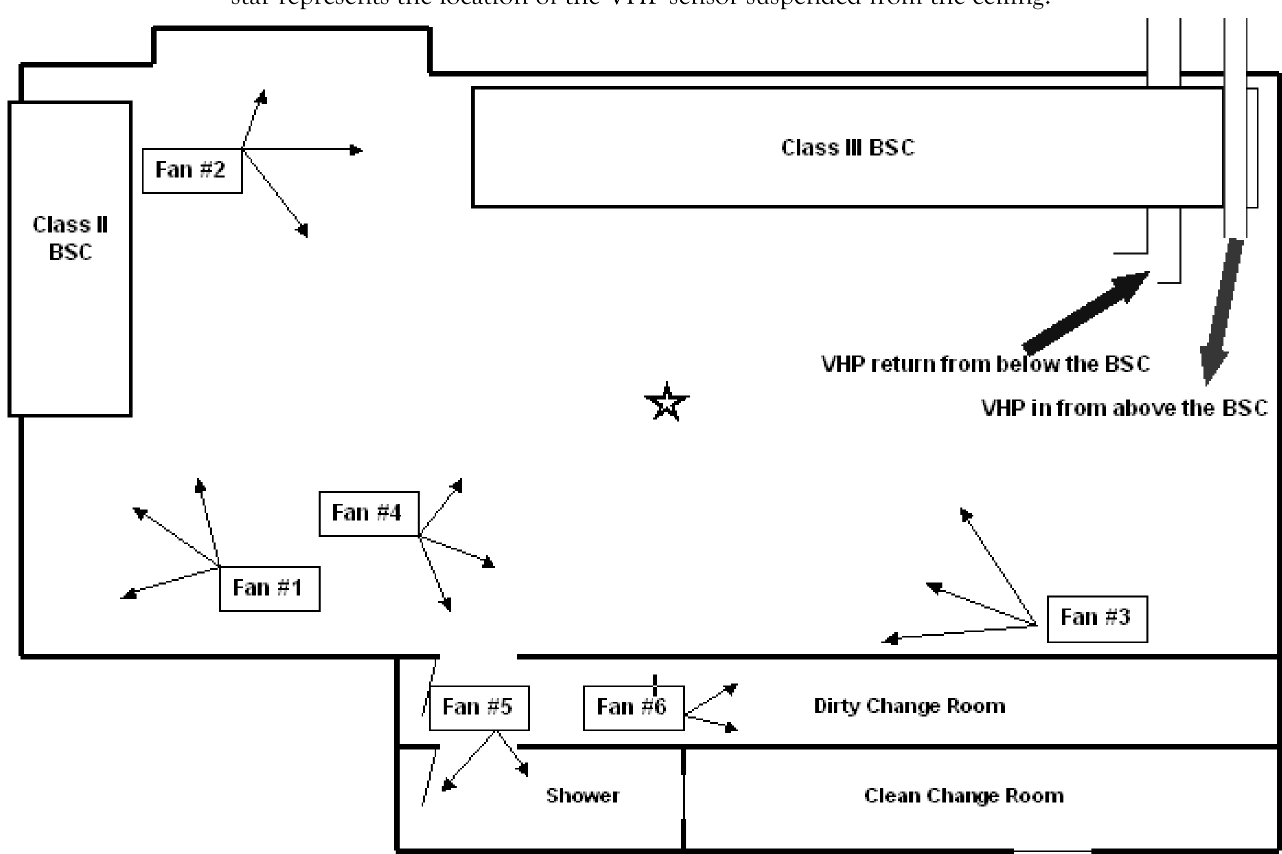
The following parameters were programmed into the VHP generator: *Dehumidification* to 30% relative humidity (30 minutes), *Conditioning* sterilant injection rate 11g of 35% H_2O_2/min (20 minutes), *Decontamination* injection rate 8g H_2O_2/min (90 minutes), *Aeration* 60 minutes and the *Flow rate* was set at 20cfm (STERIS, 2002).

Safety Assurance

The laboratory suite was not completely airtight. To prevent hazardous levels of VHP from leaking out, the lab pressure was set at approximately minus 10 Pascals. This was accomplished by shutting down the HVAC system and opening a manual bioseal damper on the laboratory's exhaust duct to vent out a small volume of airflow. During the process, air was sampled in the neighboring labs, rooms, and the penthouse mechanical space for the presence of VHP using Dräger H_2O_2 detection tubes capable of measuring as low as 0.1 ppm VHP (Drager Safety, 2005). No VHP was detected. On the following day, the HVAC system was started to aerate the lab and to decrease the VHP concentration to safe levels. To determine

Figure 1

Laboratory suite set up for VHP decontamination. The laboratory suite is approximately 3000 cubic feet in volume. Thin arrows indicate fans' directions of oscillation and the star represents the location of the VHP sensor suspended from the ceiling.



the concentration of residual VHP inside the lab, exhaust duct air was sampled using Dräger H_2O_2 tubes. After about 24 hours of HVAC-assisted aeration, the VHP concentration fell below 0.3ppm, which is well below the personal exposure level. Therefore, normal access to the lab was permitted for the retrieval of biological and chemical indicators.

Process Control and Monitoring

An ATI series B12 two-wire gas transmitter fitted with an H_2O_2 electrochemical sensor (0-2000ppm) was suspended from the ceiling in the middle of the laboratory to monitor real-time VHP concentration. A second VHP sensor was mounted in the exhaust duct to determine the amount of VHP being lost during the decontamination process and the post process residual VHP concentration. The lab pressure during the process was monitored using a digital manometer (ATE-100, Ashcroft Instruments Canada Inc. Mississauga, Ontario). The data from these monitors were logged using OM-CP process input data loggers (±25mA) (OM-CP-PROCESS110-25MA, Omega Technologies, Laval, Quebec). The temperature and relative humidity in the lab were also monitored and logged (OM-CP RFRHTEMP101A, Omega Technologies). VHP chemical indicators (NB305, Steris®, Mentor, Ohio) were placed at different locations (N=50) in the lab to visualize the extent of VHP distribution.

Sterility Validation

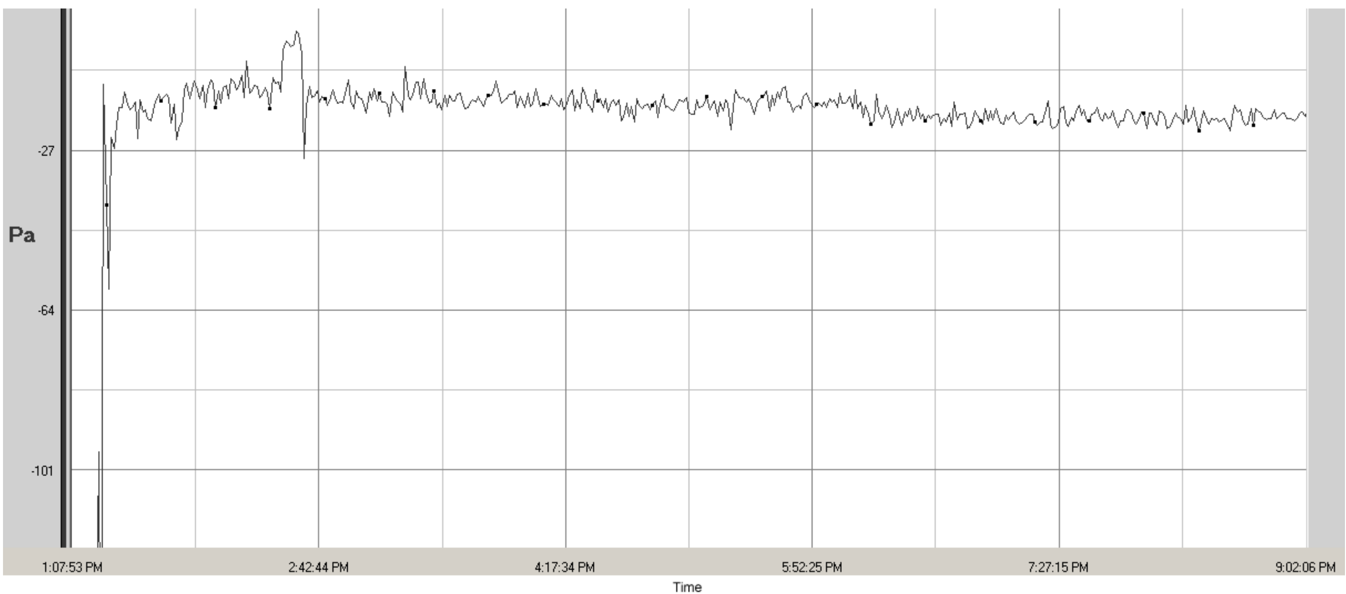
To validate the process' extent and efficiency of microbial sterilization within the lab-space, biological indicator pouches containing >106 spores of *Geobacillus stearothermophilus* dried on stainless steel metal discs sealed in Tyvek pouches (Apex Laboratories, Inc. Apex, NC) were placed, in pairs with chemical indicators, at different locations (ceilings, walls, floors, corners, and behind, under, and inside of cabinets and various equipment) within the laboratory suite (N=50). Three batches of the biological indicators were used and their lot numbers and D values were H1535 (1.6 min), H0635 (1.6 min), and H0035 (1.4 min). Upon completion of the biodecontamination program, the pouches were retrieved and opened and the discs were transferred aseptically into Tryptic Soy Broth and incubated at 56°C. An unexposed biological indicator was also included as a positive growth control. The cultures were observed for bacterial growth for up to 7 days. All cultures remained negative for growth except for the positive control, which became positive after overnight incubation.

Results and Discussion

The authors believe that this study is the first published work describing the VHP-based biodecontamination of a laboratory suite under negative pressure while the rest of the building was occupied. VHP has been in use as an alternative to formaldehyde for space biodecontamination. Following the post 9/11 anthrax letter campaign in the United States, the State Department mail-processing facility SA32 (1.4 million cubic feet of volume) was decontaminated with VHP (National Homeland Security Research Center, 2005). The mail-processing facil-

Figure 2

Laboratory's negative pressure. The laboratory suite was kept at about minus 10 Pascals to prevent VHP from leaking out into the surrounding area. This was monitored and maintained throughout the VHP decontamination process.



ity was kept at negative pressure during the decontamination to prevent VHP from escaping into the surroundings. However, this was done only after complete evacuation of the facility. An average of minus 11.28 Pascals of negative pressure (Figure 2) was maintained throughout the decontamination process by venting out 2.97 (average) cubic feet of air per minute from the lab (Figure 3). By doing so, it was anticipated that a significant amount of VHP would be lost via the exhaust air. However, VHP was not detected in the exhaust duct until 6 hours after the end of H_2O_2 injection. Possible explanations for this lag in detection of VHP by the sensor mounted on the exhaust duct include the following:

1. The volume of air preexisting in the exhaust duct (about 60 cubic feet) had to be vented out at a rate of 2.97 cubic feet per minute before the VHP-containing air reaches the sensor.
2. The sensor was placed upstream to two 24 X 24 HEPA filters and they are known to absorb and retain VHP (Jones et al., 2004).
3. The galvanized metal exhaust ductwork is capable of decomposing H_2O_2 into water and oxygen and thus diminishing small quantities of VHP from reaching the sensor.

Spores of *G. stearothermophilus* have been identified as the hardiest bacterial spores to VHP inactivation (Kokubo et al., 1998; Meszaros, 2005; Rickloff & Orelski, 1989). Therefore, we have chosen to validate our biodecontamination processes using *G. stearothermophilus* spore-discs as biological indicators. Furthermore, the biological indicators were strategically placed on locations (behind, under, and inside cabinets and equipment) that are harder for the VHP to reach. The peak VHP concentration measured at the central location of the laboratory suite was 517ppm (Figure 4), slightly higher than the concentration reported in a recent hospital ward decontamination study (French et al., 2004). However, cultures of all the biological indicators failed to grow upon incubation up to a week indicating the thoroughness of microbial decontamination achieved within the laboratory suite (data not shown). This was not surprising because a VHP concentration of less than 100 ppm was shown to be cidal to *G. stearothermophilus* spores (National Homeland Security Research Center, 2005). The authors were unable to do total colony counts of the environmental microbes before and after the decontamination processes because the laboratory was supplied with nonsterile un-HEPA-filtered air.

The authors were able to visualize most of the chemical indicators placed within the laboratory in real-time using the security camera. A few minutes after the sterilant injection, these underwent a color change from blue to beige indicating their contact with VHP. All 50 chemical indicators retrieved at the end underwent color changes indicating that VHP reached virtually everywhere within the laboratory suite.

The VHP concentration in the laboratory continued rising until the beginning of the aeration phase; then it started to decline slowly. The longest phase of the exercise was the postprocess aeration. It took over 24 hours of HVAC-assisted aeration for the VHP concentration to fall below 1ppm, the permissible personal exposure limit, whereas the actual decontamination program cycle required only 3 hours and 20 minutes. The design of the laboratory suite with its exhaust air vented out directly

Figure 3

Exhaust air flow. A slight flow of air from the lab was exhausted to maintain negative pressure in the laboratory suite. This was monitored throughout the VHP decontamination process.

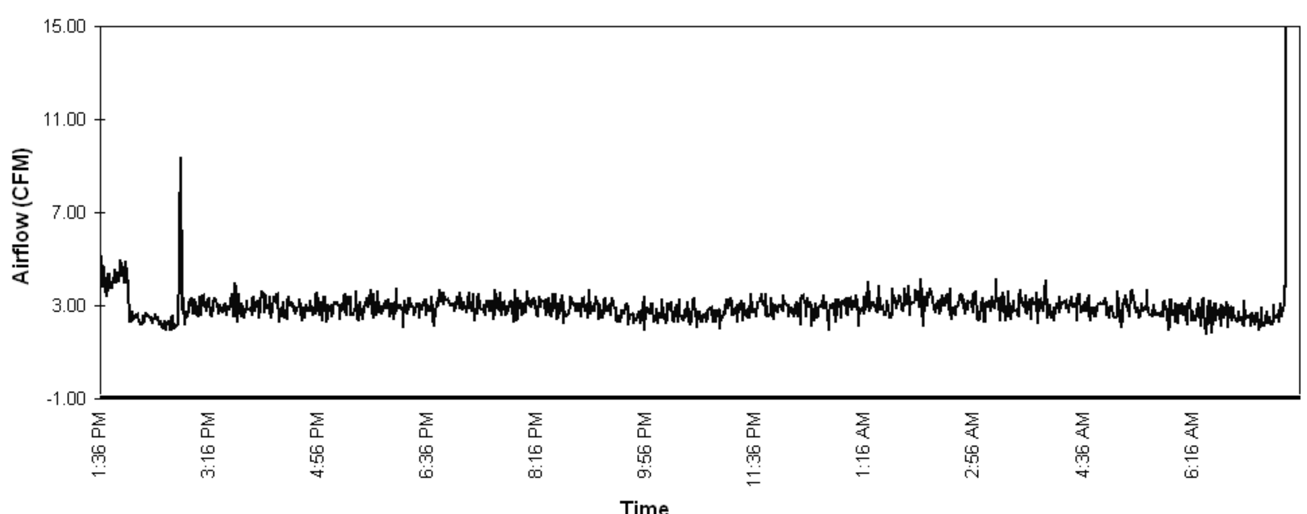


Figure 4

VHP concentration recorded in the lab (thin line) and in the exhaust duct (thick line). Note that the actual decontamination program cycle lasted only 200 minutes (boxed) and no VHP was detected in the exhaust air during this period.

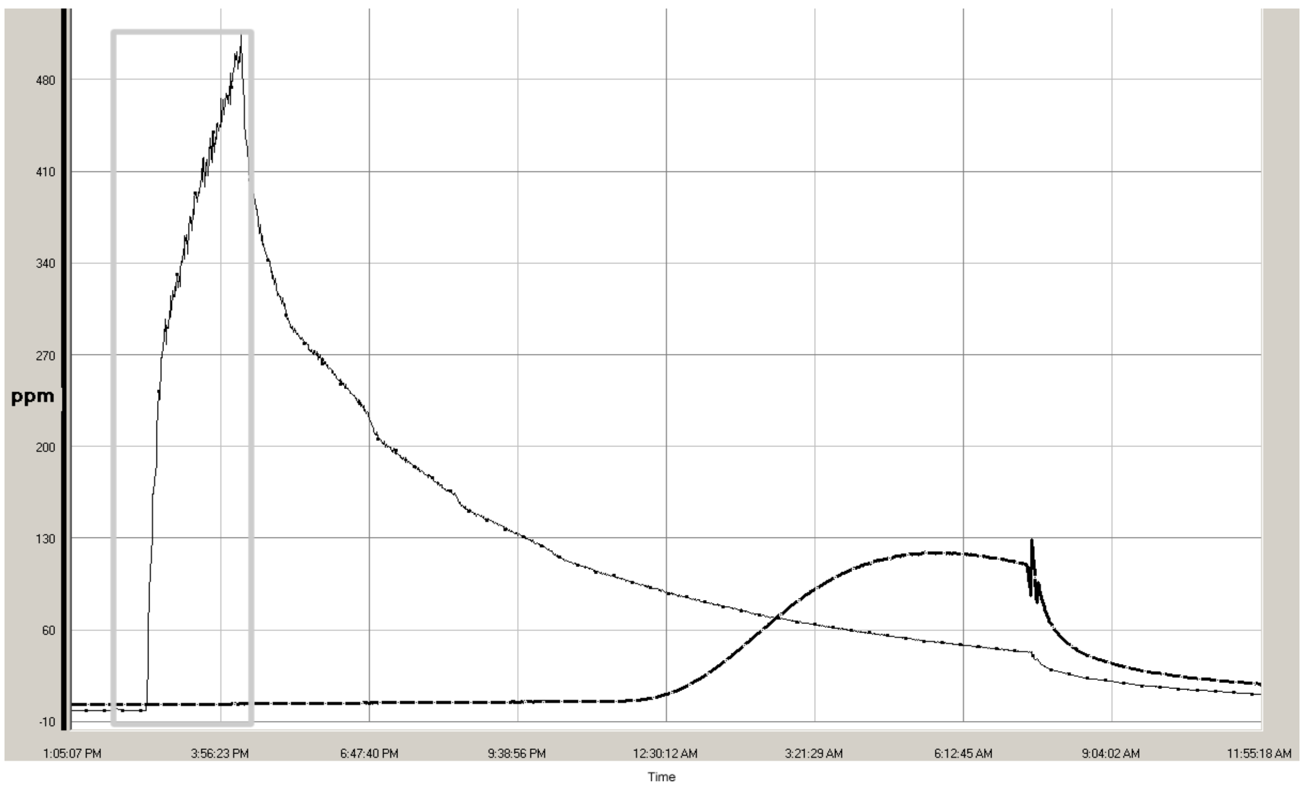
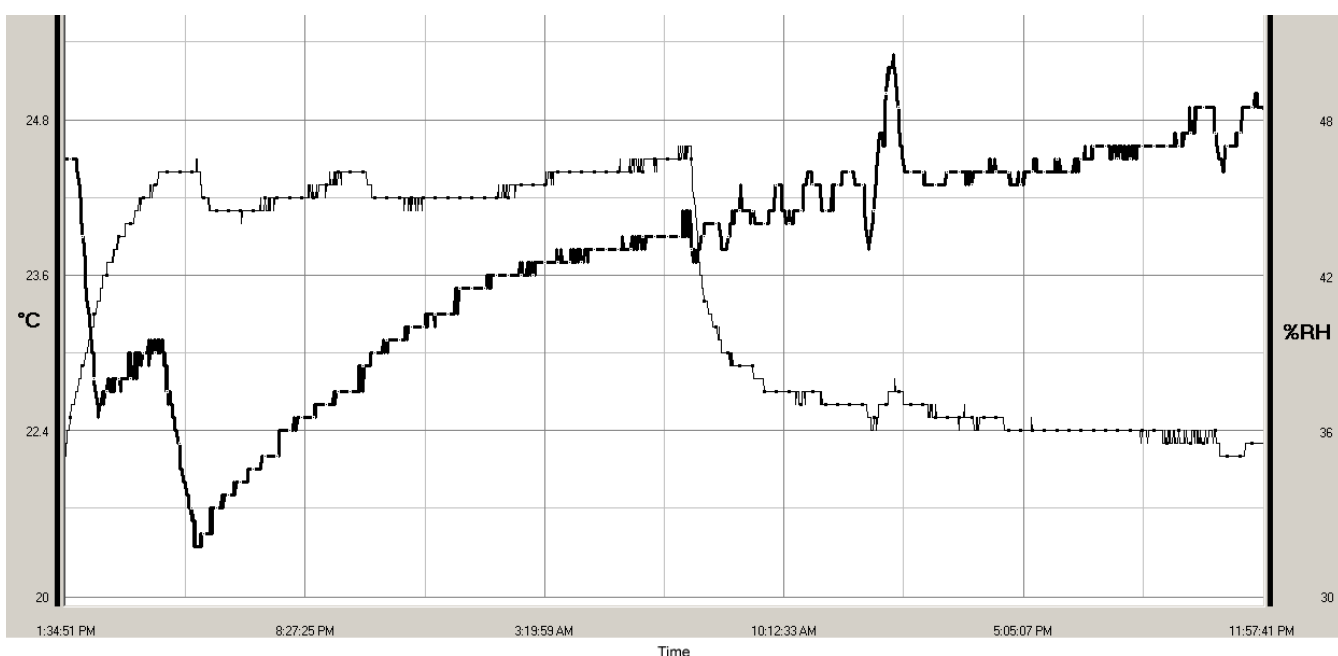


Figure 5

Temperature and relative humidity recorded in the lab. The temperature (thin line) rose from 21.3°C to 24.5°C by the end of the aeration phase whereas the relative humidity (thick line) fell from the initial 47% to 36.5% during the dehumidification phase, rebound back to 39.5% by the end of decontamination phase before falling further to 32%.



facilitated the aeration and removal of residual VHP from the laboratory. All the equipment and electronics in the laboratory remained fully functional after the biodecontamination processes (N=5).

Relative humidity directly affects the condensation of VHP; therefore, it was important to reduce the relative humidity to approximately 40% to prevent the VHP from condensing to liquid H_2O_2, which would otherwise lead to a wet decontamination process. Even though relative humidity was programmed at 30%, the authors were able to achieve only 36.5% from the initial 47% by the end of the dehumidification phase. This is not unusual when dehumidifying such a large volume. During the conditioning phase, the relative humidity started to increase and peaked at 39.5% by the end of the decontamination phase and further fell to 32% by the end of the aeration phase (Figure 5). The initial temperature in the lab was 21.3°C, which continued rising throughout the program cycle and peaked at 24.5°C by the end of the aeration phase (Figure 5). Even though the temperature was not controlled, the above-noted range of temperatures proves that the VHP-based biodecontamination can be performed under ambient conditions.

Conclusion

VHP can be used safely to biodecontaminate a laboratory under negative pressure. The negative pressure virtually isolated the lab from the rest of the facility which was occupied by hundreds of people without interruption. The VHP-based biodecontamination was auditable, reproducible, and compatible with a variety of routine laboratory equipment and electronics. The decontamination process was fast, just over 3 hours, but the postprocess aeration required more than 24 hours. In conclusion, this study shows that VHP is an effective alternative to formaldehyde for volume decontamination.

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Fact Sheets on Terrorist Attacks

The U.S. National Academies of Science has prepared fact sheets to provide reporters with reliable information on biological, chemical, nuclear, and radiological attacks. This effort was a collaboration with the U.S. Department of Homeland Security, and the Radio and Television News Directors Foundation. ABSA members may find the information useful in educational efforts on emergency planning.

The fact sheets can be found at www.nae.edu/factsheets.

Biological Attack (pdf file, 277 KB)—Where do biological agents originate? What's the difference between "infectious" and "contagious"? How long after exposure will symptoms appear?

Chemical Attack (pdf file, 72 KB)—What are the different origins of toxic chemicals that could be used? How do chemical toxicities vary? What are the practical steps to take if there's a chemical release?

Radiological Attack (pdf file, 68 KB)—What are radiological dispersal devices, a.k.a. "dirty bombs"? How are they different from nuclear bombs? What are their physical and psychological health effects?

Nuclear Attack (pdf file, 192 KB) **NEW!**—What is radioactive fallout, and how is it dangerous? What are the short-term and long-term effects of radiation exposure? What is the likely size of a nuclear explosion from an attack by terrorists?

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Biological Monitoring of Ultraviolet Germicidal Irradiation in a Biosafety Level 3 Laboratory

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Abstract

Ultraviolet germicidal irradiation (UVGI) lamps are used in biological safety cabinets and laboratory containment rooms as methods of surface decontamination. Although some organizations have discouraged the use of UVGI for disinfection, many scientists continue to request this method. The objective of this study was to assess the reliability of using UVGI produced from UV ceiling lamps as an effective method to decrease microbial agent contamination from floor surfaces in a biosafety level 3 laboratory. Actively growing Bacillus cereus *and* B. anthracis *vegetative cells or spores (Sterne strain-Veterinary Vaccine Formula) were used as biological indicators to assess UVGI effects within the containment laboratory. Studies were conducted using UVGI exposure times ranging from 15 minutes to 2 hours with varying inoculums ranging from 10^3 to 10^9 colony-forming unit per sample. Later in the study an UVGI radiometer was used to determine the UVGI intensity and to measure the correlation between the biological indicator results and mechanical instrument data. Study results showed that ceiling-mounted UVGI lamps were effective in reducing the viability of both* B. cereus *and* B. anthracis *vegetative cells and spores after a minimum UVGI exposure time of 1 hour at an intensity as low as 8 uW/cm^2. Additionally, an UVGI radiometer could be used to determine UVGI effectiveness and a UVGI intensity of 8 uW/cm^2 to 42 uW/cm^2 corresponded with the increased disinfection observed.*

Introduction

Ultraviolet germicidal irradiation (UVGI) has been shown to be an effective disinfecting or sterilizing agent against both vegetative and spore forms of bacteria, as well as other microbial agents (Blatchley et al., 2001; Dietz et al., 1980). The antimicrobial effect of UVGI is accomplished by the adsorption of energy in the Ultraviolet-C (UV-C) range of 100-280 nm (ABSA Position Paper, 2000) which chiefly affects purines and pyrimidines of nucleic acids. This alteration in nucleic acids leads to a block in transcription, thereby preventing replication of the cell. Mercury vapor lamps, also referred to as germicidal lamps, are the most common lamps used for disinfection purposes since they emit 90% of their radiation in the UV-C wavelength of 254 nm (Davis et al., 1980).

Numerous studies have been conducted to document the effects of UVGI exposure against microbial agents. These include the decontamination of air in hospital rooms (Banrud et al., 1999; Botzenhart et al., 1976; Rudnick, 2001), the disinfecting of biological safety cabinet interior surfaces (Fleming et al., 1995), and the sterilization of consumable products (Varnam, 1991). Microbial agent-specific studies using UVGI for decontamination have been reported for rhinovirus (Myatt et al., 2003), *Mycobacterium tuberculosis* (Ko et al., 2002; Nicas et. al., 1999; Riley et al., 1976), and *Serratia marcescens* (Ko et al., 2002). A recent report also found UVGI to be an effective method to inactivate organisms in the cooling coils and drip pans in the ventilation systems of buildings (Menzies et al., 2003). Recently, the Environmental Technology Verification Program was established by the U.S. Environmental Protection Agency's National Homeland Security Resource Center to test UV lights in treatment systems as a means to protect against biological contamination in buildings and other public places (www.epa.gov/etv).

Although there are many positive benefits for using UVGI, as noted above, a debate continues on the application of this technology in research laboratories (Dietz et al., 1980). The inability of UVGI to penetrate objects, problems caused by shelving and equipment shadowing, and the occupational risks of workers to UVGI exposure (Talbot et al., 2002; U.S. HEW, 1972) are the main issues that continue to draw questions about the use of this technology as a reliable and safe method for disinfection within the laboratory. In December 2000, the American Biological Safety Association (ABSA) published a position paper on the use of ultraviolet lights in biological safety cabinets (BSC) and stated that "UV lights are not recommended for use in a biological safety cabinetry." In this position paper the Centers for Disease Control and Prevention also stated that "UV lamps are not required in BSC" (ABSA Position Paper, 2000). However, since many individuals will continue to use UVGI for disinfection

purposes, methods for monitoring the effectiveness of this process will need to be available. Additionally, to advance the applications of ultraviolet technology, the International Ultraviolet Association was recently established, in part to encourage research into using this methodology (www.iuva.org). This study reports on a method for the biological monitoring of UVGI ceiling lamps for decontamination purposes in a biosafety level-3 (BSL-3) laboratory.

Materials and Methods

BSL-3 Laboratory Design and UVGI Lamp Placement

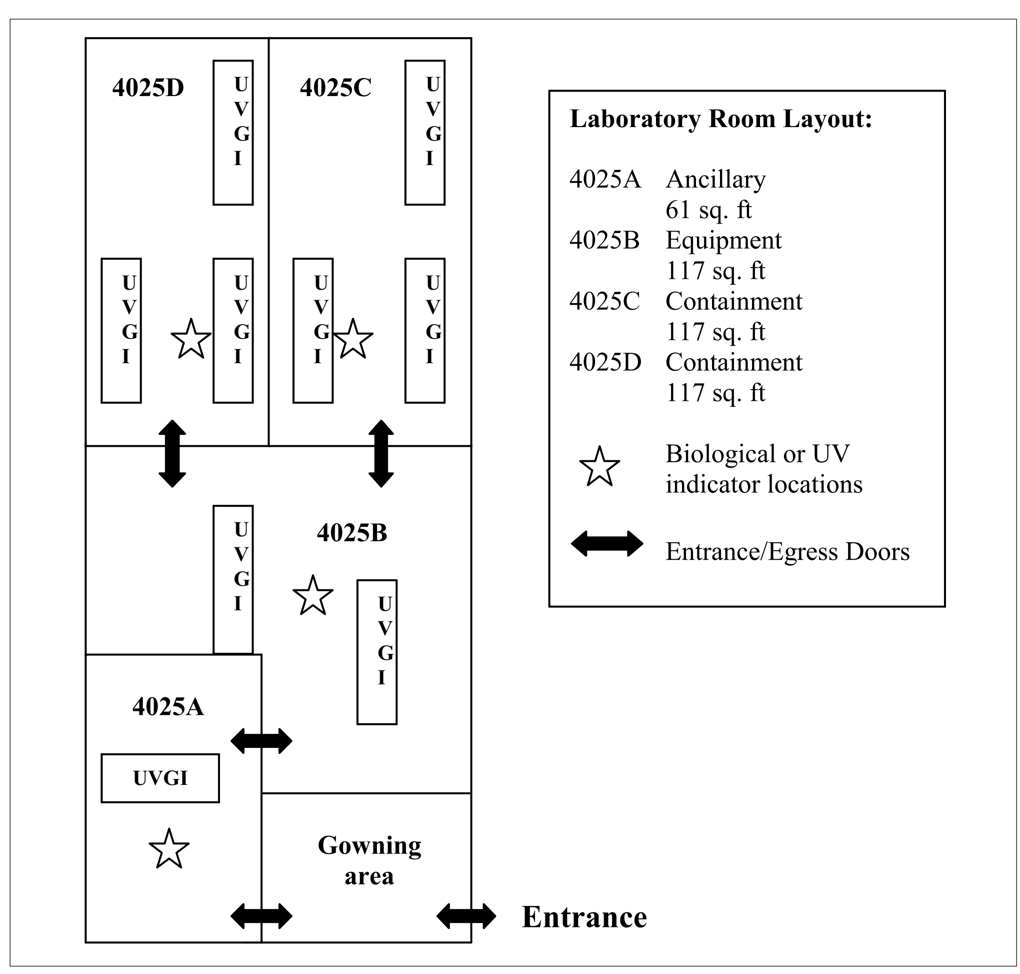
The BSL-3 laboratory evaluated was composed of four separate rooms with UVGI lamps located as illustrated in Figure 1. The UVGI lamps (Model G30T8, Sylvania Manufacturing Co.) were 36 inches long, 1 inch in diameter, and emitted 30 watts of irradiation at a wavelength of 254 nm. The ceiling height in all rooms was 9 feet. The lamps in each room were on separate timer mechanisms, which were placed on various settings ranging from 15 minutes to 2 hours. The humidity in the rooms was < 60% and the temperature ranged from 68°F to 72°F during the evaluation. Air in the laboratory was high-efficiency particulate air (HEPA) filtered, single-pass 100% outside air supplied.

Radiation Measurement

UVGI was measured using an UVX Radiometer (Ultra-Violet Products, Upland, CA) fitted with a sensor to measure irradiation at a wavelength of 254 nm. A 12-foot probe extension allowed for readings to be taken across the entire floor area in each working suite. The UVX instrument had three application ranges which cover a span from 0 uW/cm^2 to 20 mW/cm^2, 0 to 200 uW/cm^2, and 0 to 2000 uW/cm^2. The instrument was calibrated to zero before readings were taken. Readings were recorded as uW/cm^2 per manufacturer's instructions.

Figure 1

Schematic representation of ultraviolet germicidal irradiation lamp (UVGI) placement in the containment laboratory.



Biological Testing

For biological testing, cultures of *Bacillus cereus* (ATCC 86100) or *B. anthracis* (Sterne strain-Veterinary Vaccine formulation, Colorado Serum Company, Denver, CO) were grown at 37°C for 24 to 48 hours on sheep blood agar (SBA) plates (Remel, Lenexa, KS). Inoculum suspensions of the actively growing *B. cereus* or *B. anthracis* vegetative cells were made in sterile phosphate buffered saline (PBS) (Sigma Scientific Co., St. Louis, MO). The *B. anthracis* spore suspension (Sterne strain-Veterinary Vaccine formulation) was used directly from the vaccine vial. A 0.1 ml amount of each inoculum suspension was pipetted onto a SBA plate and spread using a sterile disposable inoculating loop. The inoculum was subsequently 10-fold serially diluted in PBS to determine viable bacteria per ml. For maximum UVGI exposure, the inoculated SBA plates were placed on the floor and centered directly under the germicidal lamp(s) in each room (Figure 1). This placement was necessary in rooms 4025 C and D due to equipment and shelving along the walls. The lids were removed and the plates were exposed to the UVGI for times ranging from 15 minutes to 2 hours. Additionally, unexposed control plates of the same inoculum, held at room temperature during the tests, were also prepared to determine the viable bacterial or spores per ml inoculum. Bacterial counts from exposed and control plates were tabulated after overnight incubation at 37°C in ambient air and expressed as colony forming units (cfu) per ml. Colonies that grew on the SBA plates were verified by colonial morphology and Gram stain as *Bacillus* spp. A greater than 3-$\log_{10}$ (0.1% viability) reduction in bacterial growth was defined as adequate reduction in viability following UVGI exposure. (Blatchley et al., 2001).

Results

The results of 20 months of monitoring using varied amounts of either *B. cereus* or *B. anthracis* vegetative cells as inoculum are shown in Table 1. The data showed that a 2-hour exposure to UVGI ceiling lamps in each room of the BSL-3 laboratory was effective in reducing the numbers of *B. cereus* or *B. anthracis* vegetative cells present on the exposed SBA plate in all areas. No differences in survival rates were seen between the different inoculum sizes or between *B. anthracis* or *B. cereus* vegetative cells. In all cases, colonies growing were embedded in the agar.

The effect that exposure time to UVGI had on inactivation of the *Bacillus* spp. vegetative cells is shown in Table 2. A greater than 3-$\log_{10}$ reduction of vegetative cells was noted in all areas at 15-, 30-, 60-, and 120-minute exposure periods. However, as the number of UVGI ceiling lamps in the lab rooms decreased, increased bacterial counts were noted. In no case was complete sterilization of the plates accomplished.

Table 3 shows the comparisons of a 1-hour ultraviolet germicidal irradiation (UVGI) exposure (as determined by radiometric readings) for effects on *B. anthracis* vegetative cells of varying inoculums size. As the data indicate, no significant difference was seen in the bacterial viability between the two inoculums tested. Table 4 shows a direct comparison of *B. anthracis* vegetative cells and *B. anthracis* spores to UVGI exposure (as determined by radiometric readings). These comparisons showed that with both vegetative cells or spores used as biological indicators, the cumulative effects of UVGI intensity generated over a 1-hour time period and thus the total disinfectant effect created were dependant upon the number of germicidal ceiling lamps located in each room.

Discussion

The ability to monitor the disinfecting properties of ultraviolet germicidal irradiation (UVGI) using a biological method would be a useful tool for those who utilize a biosafety level 3 (BSL-3) laboratory. Fleming et al. (1995) have shown UVGI lamps to be effective to sterilize exposed surfaces of the BSC, when using the proper controls. More recently, First et al. (2005) demonstrated the safety of personnel working in rooms with UVGI in place and being utilized above a 6.5-ft. height. The present study examined the feasibility and reliability of using a biological method to monitor UVGI as a method to decrease bacterial contamination from floor surfaces in a BSL-3 laboratory while using ceiling-mounted UVGI lamps.

The proper placement and number of lamps installed in each room of the containment laboratory were important considerations during the design process. This lamp placement, along with determination of adequate exposure time to UVGI, was needed so that effective irradiation levels could be accomplished. UVGI over a 2-hour exposure time was shown to be effective in decreasing vegetative *B. anthracis* and *B. cereus* cells in all rooms of the BSL-3 laboratory at the locations studied. Further studies showed that a 1-hour UVGI exposure time would suffice in all BSL-3 laboratory rooms within a well-defined exposure area, but that an increased exposure time in the room having one lamp might be considered to ensure adequate disinfection.

As expected, UVGI effectiveness increased as exposure time increased, and the reduction in bacterial and spore viability was more pronounced in rooms containing three lamps compared to those with two or one lamp(s). Direct comparison between the biological indicators and radiometric measurements showed that the intensity of the UVGI was dependant upon the number of germicidal lamps installed in each room and decreased accordingly, as might be expected, with fewer lamps present. The direct comparisons allowed for utilization of the radiometer alone for future measurements of the germicidal activity

Table 1

Biological testing to show effectiveness of ultraviolet germicidal irradiation using various bacterial vegetative cell concentrations.

Bacillus spp. concentration (median, range) [c]	Testing frequency [d]	Number Positive samples/room [b]			
		4025A	4025B	4025C	4025D
1.25×10^3 (5×10^2 to 2×10^3)	11	2	2	2	1
2×10^6 (1×10^6 to 3×10^6)	2	1	0	0	0
3×10^7 (2×10^7 to 4×10^7)	5	3	2*	3	3**
3×10^8 (2×10^7 to 4×10^9)	2	1	1	1	0

a Study was conducted over a period of 20 months using an exposure time of 2 hours.

b Number of samples showing at least one colony or more. One plate was placed into each room per evaluation. The colony counts per plate for positive results were < 30 cfu/ml. In all cases, colonies growing were embedded in the agar.

c Colony forming units/ml was determined from positive control plates. Both *B. cereus* and *B. anthracis* vegetative cells were used in the evaluation.

d Number of times testing was done at this inoculum level over the 20-month period. Radiometic readings were not taken during this time period.

* One of the lamps failed to activate.

** These evaluations were not done.

Table 2

The effect that exposure time had on the inactivation of vegetative *Bacillus anthracis* or *B. cereus* cells following ultraviolet germicidal irradiation exposure.

Room No.	No. Lamps	Colony Counts [a]			
		15[b]	30[b]	60[b]	120[b]
4025 A	1	>300	>300	100	5[c]
4025 B	2	>300	100	15[c]	2[c]
4025 C	3	>300	41	1[c]	5[c]
4025 D	3	>300	30	5[c]	9[c]

[a] One plate was placed into each in each room per evaluation. Expressed in colony forming units (cfu) per plate. The inoculum used was 3.4×10^6 cfu/ml.

[b] Exposure time expressed in minutes.

[c] Colonies embedded in agar.

Table 3

Comparison of the effects of a 1-hour ultraviolet germicidal irradiation exposure on *B. anthracis* vegetative cells of varying inoculum sizes.

Location	No. Lamps	Inoculum [a]	Energy [b] Reading	Colony Counts
4025A	1	1	8	5
		6	13	13[c]
4025B	2	1	14	4
		6	26	3
4025C	3	1	26	1
		6	31	0
4025D	3	1	25	1
		6	35	0

[a] Inoculum count x 10^7 cfu/mL per sample
[b] Ultraviolet germicidal irradiation energy readings were taken at 254 nm and recorded in *u*W per cm^2.
[c] Colonies growing were embedded in the agar.

Table 4

Comparison of the effects of ultraviolet germicidal irradiation intensity on the inactivation of B. *anthracis* vegetative cells and spores.

Lab Room	Number Lamps	Energy Reading [a]	Counts in cfu/ml [b, c]	
			Cells	Spores
4025A	1	12	> 300	> 300
4025B	2	26	> 300	~ 300
4025C	3	34	~ 300	135
4025D	3	38	112	68

[a] UVGI readings were taken at 254 nm (recorded in *u*W per cm^2) during a 1-hour exposure time.
[b] In colony forming units (cfu)/plate. Inoculums were: 2 x 10^8 cfu/mL for *B. anthracis* vegetative cells and 7 x 10^7 cfu/mL for *B. anthracis* spores.
[c] Counts are an average of two plates per room per inoculum.

on the UVGI ceiling lamps. An UVGI reading as low as 8 uW/cm^2 during a 1-hour exposure was adequate to produce a greater than 3-log_{10} reduction in viable bacteria. Since the intensity of UVGI emitted decreases with use over time, periodic use of biological indicators or radiometric measurements will be needed to ascertain the effectiveness of the lamps throughout the course of utilization.

Since UVGI is a known exposure danger, and there are limitations when using this method, any laboratory proposing to use UVGI ceiling lamps should make certain that proper built-in safeguards and safety precautions are present. Known dangers of UVGI exposure to the eyes and skin are well documented (ABSA Position Paper, 2000; Talbot et al., 2002; US HEW, 1972). The Clinical Laboratory Improvement Act (CLIA) general checklist (Gen 70832, Phase 1) now requires that use of UVGI lamps be evaluated by asking the following question: "Are policies documented to prevent or reduce UVGI light exposure from instrument sources?" In the BSL-3 laboratory, a laboratory-specific UVGI Safety Plan should be initiated to reduce the danger of accidental UVGI exposure to laboratory personnel. Suggestions for a plan include:

- Posting of "Caution—UV ceilings lamps in use" warning signs
- Placement of UV-protection film on all door glass
- Installation of "lock-out" light switches so that when the regular lights are turned "on," the UV lamps are not activated
- Installation of door electrical connections so that when the door to a room is opened the UV lamps are deactivated
- Mechanisms for proper disposal of germicidal lamps containing mercury vapor
- A well-written, laboratory-specific Standard Operating Procedure (SOP) for the utilization of these lamps. The SOP should outline parameters such as the cleaning, monitoring, maintenance, and replacement of the UVGI lamps; timer settings for exposure lengths (minutes or hours); and when (end of day or between room uses), by whom (laboratorian or facilities manager), and how often (daily or weekly) the UV lamps should be activated.

Likewise, these safety parameters should be considered when utilizing UVGI in biological safety cabinets.

Conclusions

This study showed the effectiveness of UVGI exposure on reducing bacterial cell counts on exposed floor surfaces in a BSL-3 containment laboratory. Effectiveness was dependant upon both the number of UVGI lamps in each room and on the exposure time used. A 1-hour exposure time with a measured UVGI intensity as low as 8 uW/cm^2 resulted in a greater than 3-log_{10} reduction (0.1% viability) in viable bacteria. As a result of this study, location-dependant exposure times are now included with the Standard Operating Procedures for the laboratory. When UVGI effectiveness decreases below that of the predetermined cut-off rate of 3-log_{10} reduction in plate counts, the lamps should be cleaned (if found to be dirty) or replaced. Utilization of UVGI should not be considered as a replacement for para-formaldehyde or other accepted decontamination methods for a biological safety cabinet or a laboratory room. Additionally, as a supplement to UVGI, weekly disinfection of the floors with a fresh solution of 10% bleach or other disinfecting solution should still be considered.

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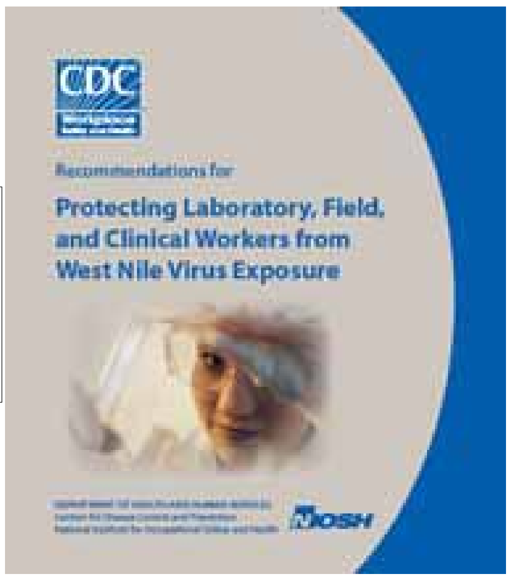
NIOSH Guide

NIOSH has an excellent new guide available for the prevention of occupational exposures to West Nile virus.

www.cdc.gov/niosh.docs/2006-115/#d



Safety Considerations for Handling Imported Fire Ants (*Solenopsis spp.*) in the Laboratory and Field

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Abstract

Imported fire ants pose two primary threats to humans: the risk of anaphylaxis following stings, and the potential for secondary infection of localized pustules that result from stings. Additionally, fire ant products such as whole-body extract and venom can present risks to laboratory personnel. Whether in concentrated form in the laboratory or retained within the living animal, fire ant venom alkaloids and proteins are biological toxins with potentially severe health effects. Several recommendations are given for minimizing the risk of stings in the laboratory and field with the understanding that most experienced workers are capable of assessing their own tolerance to stings and acting accordingly. Since there are no fail-safe methods for avoiding fire ant stings, all workers who are exposed to imported fire ants should be familiar with the symptoms of anaphylaxis and be prepared to seek immediate medical assistance if they or their coworkers are stung and show symptoms of hypersensitivity.

Introduction

Imported fire ants are serious pests to humans and livestock in the southern United States (Vinson, 1997). They were unintentionally imported in cargo ships from South America early in the 20th Century (Lofgren, 1986) and have since spread to infest more than 300 million acres in the U.S. (Figure 1) (USDA, Animal and Plant Health Inspection Service, 2005). Current infestations are likely to expand to the north, and the entire West Coast is susceptible to invasion (Korzukhin et al., 2001). The red imported fire ant (*Solenopsis invicta* Buren) infests the southeastern U.S. from coastal North Carolina to west Texas, with additional infestations in New Mexico and California. Red imported fire ants have also been found in several sites throughout the West Indies (Davis et al., 2001) and were introduced into Australia in the late 1990s (McCubbin & Weiner, 2002) and more recently into Taiwan (CNA News, 2005) and mainland China (China Daily, 2005). The black imported fire ant (*S. richteri* Forel) is currently limited to an area of several counties in northeastern Mississippi and northwestern Alabama (Shoemaker et al., 1994), and south-central Tennessee. A hybrid of the two species also exists in the U.S., occupying a broad band from western Mississippi through Alabama and parts of Georgia (Shoemaker et al., 1994). Red, black, and hybrid imported fire ants exhibit similar aggressive tendencies and stinging behavior and will hereafter be referred to as "imported fire ants."

Imported fire ants reside in colonies of 250,000 or more individual ants, each equipped with strong mandibles and a potent sting. They typically live in the soil in large, earthen nests (commonly referred to as mounds) (Figure 2A) that are created as they excavate underground galleries; however, they can also nest most anywhere sufficient moisture and favorable temperatures are found. Nests are often cryptic, occurring under or within stored equipment, in wall voids (Figure 2B) or rotten wood (Figure 2C), and inside electrical or telephone junction boxes (Figure 2D).

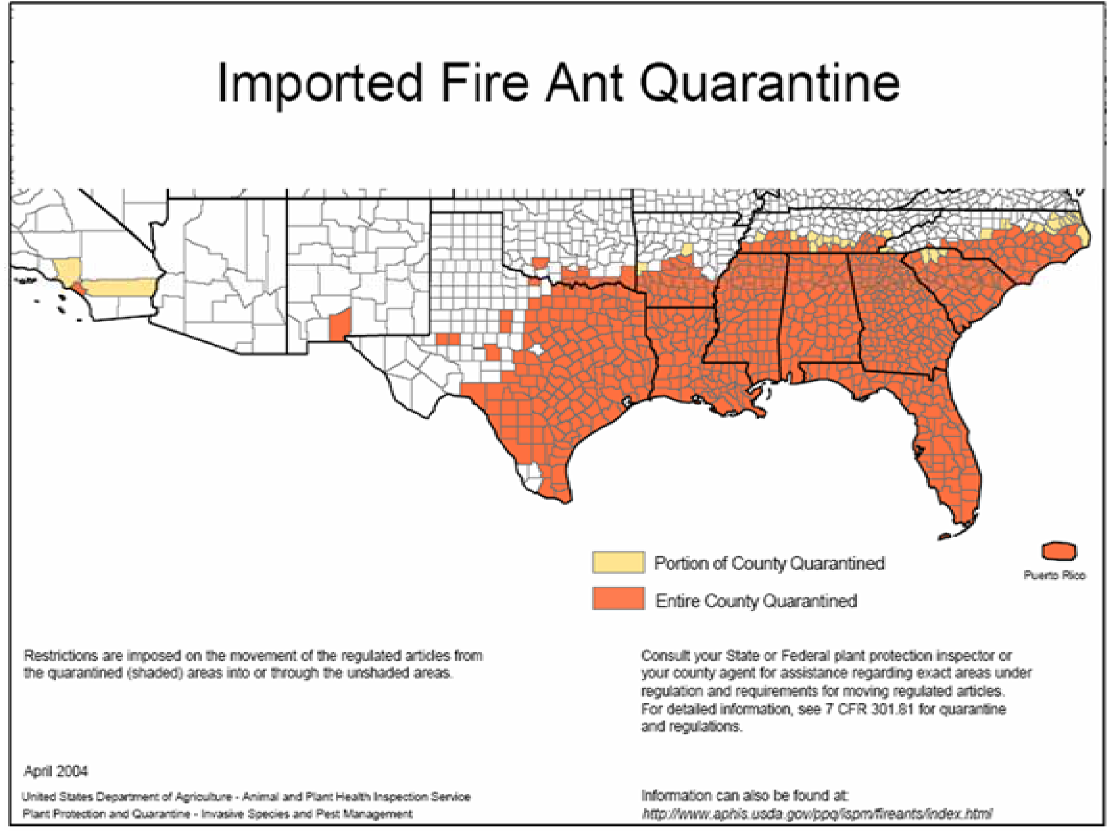
When an imported fire ant worker stings, she first gets a firm grip with her mandibles, then arches her body pointing the gaster downward and stings repeatedly. The intense burning sensation that occurs when the venom is injected accounts for the popular name of "fire ant."

Clinical reaction to the sting can range from localized pustules (Figure 3) to life-threatening anaphylaxis (reviewed by Stafford, 1996). Anaphylaxis is relatively rare but may be very serious when it occurs. The domestic species *S. xyloni* McCook and *S. geminata* (Fab.) are known to have caused serious and fatal reactions (D. R. Hoffman, personal communication), but are much less abundant than the imported fire ants. Sting victims sometimes suffer tens or hundreds of stings, especially in cases where the victim is immobile due to sickness or injury. Unsensitized people can rapidly develop imported fire ant-specific IgE following limited stinging events (Tracy et al., 1995), which can lead to hypersensitivity and the potential for anaphylaxis to occur.

Imported fire ant venom is unlike bee or wasp venom in its composition. It is composed of >95% piperidine alkaloids (MacConnell et al., 1971) and a small amount of several proteins. Piperidine alkaloids are identified by their saturated heterocyclic ring (i.e., piperidine nucleus).

Figure 1

Map of imported fire ant infested areas in the United States (courtesy of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service).



The best known piperidine alkaloids are those of poison hemlock, *Conium maculatum* L. Some imported fire ant venom alkaloids have been shown to cause cardiorespiratory depression in experimental animals (Howell et al., 2005). The protein component of the venom is responsible for the development of hypersensitivity. Additionally, cross-reactivity with bee and wasp venom has been demonstrated (Hoffman et al., 1988), so people who are hypersensitive to other stings should avoid imported fire ants. A recent review of the clinical aspects of imported fire ant allergy can be found in Reichmuth and Lockey (2003).

Evidence demonstrates that the more frequently beekeepers are stung, the less common their severe allergic reactions become, but presence of bee venom-specific IgE in beekeepers who are frequently stung and do not experience allergic symptoms suggests that anaphylaxis may still be possible in these individuals (Bousquet et al., 1984). It is possible that frequent imported fire ant stings may serve to desensitize individuals to fire ant venom, but no clinical data support or refute this, and the venom dose received during a fire ant sting differs by several orders of magnitude from the dose received during a bee sting (D. R. Hoffman, personal communication).

In short, imported fire ants produce biological toxins as defined by Kozlovac and Hawley (2005); the alkaloids have broad biological activity and can act as cardiodepressants, and proteins are potential allergens. As delivery mechanisms for these biological toxins, imported fire ants can be considered a hazard in the laboratory and field, and appropriate steps should be taken to reduce the risk of stings for those individuals performing field or laboratory research involving these insects or preparations of fire ant venom.

Decades of research on these pests have generated thousands of published papers, and several laboratories around the world continue to maintain stock colonies for research purposes and in-vivo rearing of parasitoids and diseases. Ongoing field research in imported fire ant-infested areas exposes workers to the potential for stings from the ants. For laboratory tasks such as colony maintenance, supervisors should develop and implement effective standard operating procedures (SOPs) to reduce the probability of stings or venom exposure, and train employees. Both supervisors and workers must be aware of the potential health hazards posed by stinging incidents or venom exposure.

This article represents a synthesis of current knowl-

Figure 2

(A) Imported fire ant mound in pasture (James T. Vogt, U.S. Department of Agriculture). (B) Loose soil is the only evidence of this fire ant nest in a wall void. Quarter shown for scale. (C) Old fire ant nest in rotting wood. (D) Imported fire ants have carried soil into electrical junction box and are nesting in the box. Note: Photos B, C, and D courtesy of Oklahoma State University Department of Entomology and Plant Pathology.

Figure 2A

Figure 2B

Figure 2C

Figure 2D

edge and recommendations for minimizing imported fire ant stings and allergen risks. Its goal is to provide useful suggestions for supervisors and workers alike as they develop work habits that adequately address imported fire ants and the risks they pose. This paper is not meant to be a comprehensive review of the medical impacts of imported fire ant stings; for that the reader is referred to the recent review by Kemp et al. (2000) and references therein.

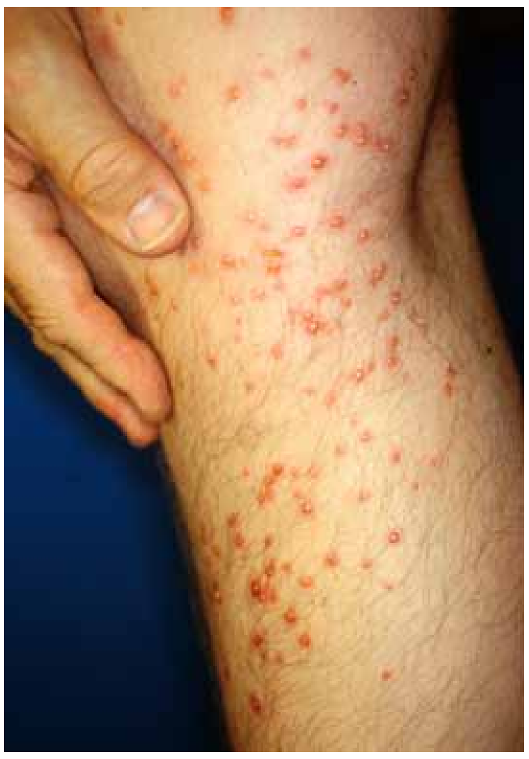
Four subject areas are addressed here: identifying imported fire ants, laboratory equipment and personal protective equipment (PPE), best practices in the field, and actions to consider if stung.

Listserv Survey

The authors conducted a search of the literature, including Extension fact sheets, to collate available information and recommendations on safe practices for handling and/or working with imported fire ants. The Cooperative Extension Service, established in 1914 by the Smith-Lever Act, constitutes one of the largest adult education programs in the world, organized at the federal, state, and county levels in the United States. Additional recommendations came from the experience of the first author, who has more than 12 years of experience in fire

Figure 3

Aseptic pustules following multiple fire ant stings
(D. P. Wojcik, Retired, and S. D. Porter, U.S. Department of Agriculture).



ant research and education. Background and general information on PPE, toxins, and safety can be found in Kozlovac and Hawley (2005) and Johnson, Mastnjak, and Resnick (2000). Hoffman (2003) provides an excellent review of Hymenoptera venoms. Finally, an informal poll was conducted among fire ant specialists who subscribe to a popular imported fire ant e-mail listserver. The following questions were sent to participants:

1. Do you have formal SOPs for procedures involving imported fire ants?
2. Do you require PPE (personal protective equipment) for working with imported fire ants in the lab?
3. If your answer to #2 is "yes," please list the PPE.
4. What safety recommendations do you have for:
    a. Maintaining/containing colonies?
    b. Day to day activities (separating brood, etc.)?
    c. Collecting in the field?
    d. Action to take if stung?

Questions 3 and 4 were intentionally open-ended. We tallied numbers 1 and 2 as a percentage of participants answering "yes" or "no," examined the number of unique answers to numbers 3 and 4, and constructed a frequency distribution to determine the most adopted safety measures.

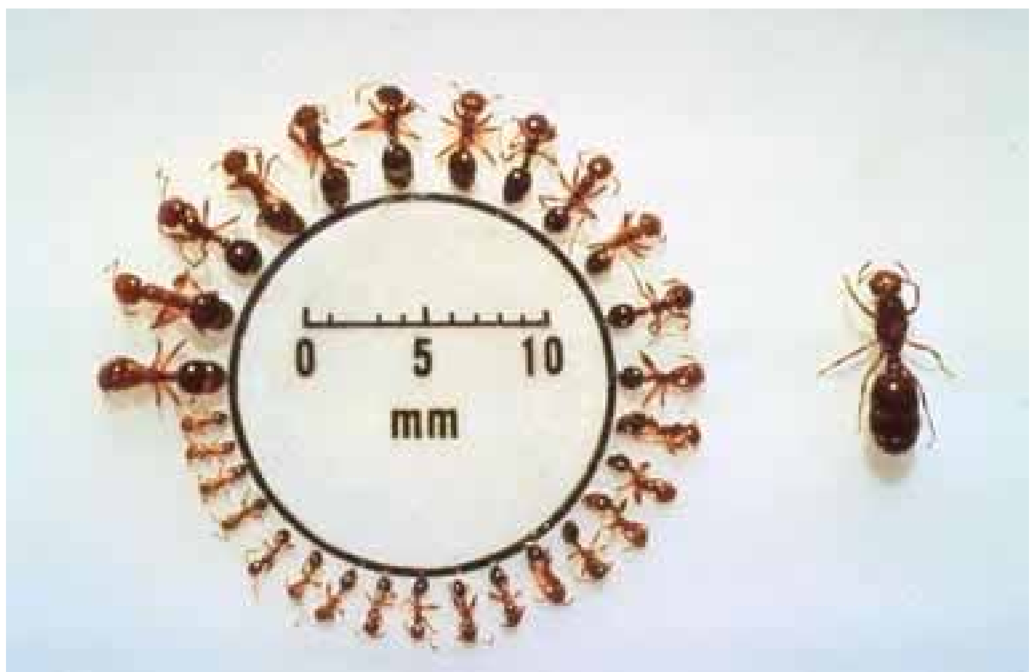
Identifying Imported Fire Ants

Consultation with a taxonomic expert is necessary for positive identification of imported fire ants to species level. This is currently accomplished using morphological structures (Wojcik et al., 1976) or chemotaxonomic techniques to examine cuticular hydrocarbon profiles of the ants (Vander Meer et al., 1985). For the layperson, general knowledge of the ants and their nest characteristics is sufficient to make a tentative identification, and formation of a characteristic pustule (Figure 3) 12 to 24 hours after a sting is almost certain evidence that fire ants were responsible. Even without a formal identification, supervisors and workers should know whether imported fire ants are present in the area where they are working and take appropriate precautions. This is especially relevant if a person who is unfamiliar with imported fire ants is moving into an infested area.

Imported fire ants are polymorphic; that is, worker size increases on a continuum from about 2.5 mm to 6 mm in length. This is important, as most ant species are either monomorphic (workers are all one size) or dimorphic (there are two distinct size classes of workers). Workers are reddish-brown to nearly black in color (Figure 4). For the reader with experience in insect taxonomy, the

Figure 4

Imported fire ant workers array illustrating size range of individuals. The large ant to the right is a reproductive queen (S. D. Porter, U.S. Department of Agriculture).



fire ant antenna is diagnostic for the genus *Solenopsis*, with 10 segments including a 2-segmented club.

Imported fire ants build characteristic mounds that can reach > 1.5 m in diameter and > 1 m in height (Figure 2A). With the exception of brief periods (usually following rainfall) when male and female winged imported fire ants leave the nest to mate and workers repair the mound surface, the mound is closed to the environment with no entrance/exit hole(s) visible. Foraging ants enter and exit the mound underground, in a series of foraging tunnels that branch out through their foraging territory in such a way that an ant generally does not have to travel above ground for more than about 0.5 m to reach any point in the colony's territory (Markin et al., 1975). A single fire ant colony can occupy a relatively large area. Foraging territories of up to 29 m^2 have been documented using radiotracers (Showler et al., 1990), and territories of nearly 200 m^2 have been documented in pasture (Adams, 2003). Obviously, a person working in an infested area is constantly at risk of being stung even if he or she avoids mounds. It is also important to note that people working or walking through vegetation may risk stings, since fire ants readily forage on plants.

Laboratory Equipment and PPE

The first problem confronting fire ant researchers who maintain indoor colonies is keeping them confined in buckets or trays. This is usually accomplished by painting or swabbing a Teflon emulsion on the inner sides of the container (e.g., Banks et al., 1981). One product that works well is Fluon® PTFE aqueous dispersion resin (AGA Chemicals Americas, Inc., Bayonne, NJ). The material can be applied in several different ways, but is commonly applied by painting it onto the surface with a sponge brush, taking care not to apply too much or leave bubbles on the surface. With some practice this technique can be used to apply a thin, even layer. The material should be applied in one direction with minimal pressure on the sponge brush to avoid bubbling. The inner surface of the container should be clean and smooth. Some important points about Fluon®:

- Consult the MSDS for appropriate PPE and precautions, including gloves. Avoid dermal contact.
- Clean up spills of wet or dry material to avoid spreading it outside the work area.
- Fluon® is an inhalation hazard when burned.
- Fluon® is not effective at high humidity.
- Care must be taken not to scratch or abrade coated surfaces.

A light dusting of talcum powder on a surface can also prevent imported fire ants from climbing container walls for several days, depending on the number of ants involved. Imported fire ants can easily climb most untreated vertical surfaces and can chew through nonmetallic materials. Electric barriers have also been used to keep

imported fire ants in containers (Howell et al., 1982; Markin, 1968). Petroleum jelly and mineral oil can also be effective at preventing the ants from climbing surfaces for a period of time.

Certain preparations can be made in case fire ants escape the laboratory. We recommend keeping talcum powder handy everywhere imported fire ants are housed or transported for experiments or rearing. If ants breach a Fluon® barrier, a dusting of talcum powder on that surface will contain them long enough for a new container to be prepared. Having a vacuum handy to rapidly collect large numbers of ants, in case part or all of a colony escapes, is also a good idea. (It should be a bagless vacuum, with filter, that is easily cleaned out.) Alternatively, some researchers prefer to keep a broom and dustpan handy for minor escapes. If imported fire ants are housed in a room that is close to sensitive areas where ant escape could result in serious consequences (near other insect cultures, sensitive equipment, etc.), a multilayered containment system is recommended. Such a system might consist of:

1. Fluon® barrier on inside of rearing containers
2. Containers placed on a table or rack with legs immersed in small containers of soapy water, or placed directly in larger pans with soapy water (e.g., Khan et al., 1967). (Oil or talcum powder could be substituted for soapy water.)
3. Sticky insect trapping compound applied around doorframes and across thresholds.

Live ants can be disposed of by dumping them into a bucket of soapy water.

When working with imported fire ants in the laboratory, the following PPE is recommended:

- Laboratory gown or apron
- Latex, nitrile, neoprene, or dishwashing gloves
- Long pants
- Socks
- Shoes (no open toe shoes)

Gloves that fit snugly to give ants fewer folds and creases to grip and that facilitate brushing the ants off the hands are recommended. Household dishwashing gloves are more effective because of the longer cuffs. A light dusting of talcum powder on the gloves will also help prevent the ants from climbing onto an individual's hands (Banks et al., 1981). When handling plastic laboratory equipment with gloves, static electricity can become a problem. Static charges on trays containing imported fire ants can make the ants difficult to handle, causing them to literally fly out of the tray and onto workers. The use of nonstatic gloves or an antistatic gun or grounded mat can minimize problems. Appropriate PPE (e.g., gloves, eyewear, etc.) will depend on the exposure potential of the specific operation.

Small insects are frequently collected using aspirating devices. As a safety precaution, avoid typical mouth aspirating apparatuses for collecting large numbers of imported fire ants (imported fire ants release exocrine gland products when disturbed). Instead, use blowing aspirators, electric aspirators, or negative-pressure aspirators—such as an aspirator connected to a variable-rate valve, and in turn connected to a tank hooked to a vacuum pump. A similar system is described in Banks et al. (1981).

It is a good idea to check clothing periodically to locate imported fire ants before they reach bare skin, noting that imported fire ants are most easily seen against a light-colored background (e.g., white lab gown). Recommendations regarding PPE are given with the understanding that laboratory personnel who are practiced handling imported fire ants have different tolerances for stings. Supervisors may wish to provide gloves but not require workers to wear them, especially for tasks that require a great deal of dexterity, such as microscope work or sorting individual ants.

Training new personnel in effective handling procedures for imported fire ants is essential. Standard procedures for separating life stages of the ants (Banks et al., 1981), rearing procedures for parasitoids (e.g., Vogt et al., 2003), and other day-to-day operations require a certain degree of dexterity and some practice. It is recommended that new workers "shadow" a trained worker for a period of time to become familiar with all aspects of laboratory procedures, including safety and SOPs. Workers must be mindful of loose clothing, jewelry, and long hair, all of which provide easy routes for imported fire ants to reach the skin if dangled into trays or buckets containing ants (Drees & Ellison, 2002).

Chemical ecologists, biochemists, and immunologists may store and handle varying quantities of imported fire ant venom or whole-body extract. Currently, no commercial sources for imported fire ant venom exist, but whole-body extract is available and is used in diagnosis and immunotherapy (Hoffman, 2003). Care should be taken to avoid aerosolizing solutions to avoid sensitization by inhalation. Although fire ant venom is not commercially available, some laboratories may obtain small amounts of lyophilized material for research purposes; it is important to keep containers tightly sealed to avoid inhalation of material (D. Hoffman, personal communications).

Best Practices in the Field

Hypersensitivity to fire ant venom can occur in an individual at any time after prior sensitization. Field personnel should be issued cellular phones so that they can contact emergency services if necessary. Also, close attention should be paid to where field vehicles are parked, and care should be taken to avoid being stung while exiting the vehicle, in case an imported fire ant mound was disturbed as the vehicle was parked. Vehicle interiors also

should be free of food wrappers, soft drink cans, and any other food sources that might attract foraging ants, which are often called "lard ants" or "grease ants" due to their attraction to greasy foods and sweets. Invasion of vehicles by foraging imported fire ants is a rare occurrence, but if a food source is detected by the ants, several hundred foraging ants can be in a vehicle within a relatively short (2 to 3 hours) time frame (JTV, personal observations). Barr (2003) advises extra caution around picnic sites and restrooms, roadsides (changing a tire or sightseeing), garbage receptacles, and fallen limbs or objects on the ground. With the exception of roadsides, these are areas where imported fire ants may find water, food, and/or structures that may conceal nests.

Clothing should include closed-toe shoes or boots, and socks. The longer the socks, the more time a worker will have to see imported fire ants crawling up the legs before they reach bare skin. Some people prefer to tuck their pants legs into their socks to reduce the ants' access to bare skin and to direct them to areas where they can easily be brushed off the clothing. Wearing socks helps delay stings (Jerome Goddard, personal communications). Unfortunately, no repellents are known to be effective at deterring attacking imported fire ants (Jerome Goddard, personal communications). Workers should watch where they step and check their lower body occasionally to be sure they are free of ants.

Latex, nitrile, neoprene, or dishwashing gloves may be worn in the field for tasks that involve handling imported fire ants. Talcum powder can be lightly dusted on any field equipment used to collect or handle ants—for example, if using a shovel to dig up ant colonies, dust the lower part of the handle to prevent ants from climbing (Banks et al., 1981; Drees & Ellison, 2002). If imported fire ants do get on the skin and begin biting and stinging, it is important to understand that they cannot be shaken or rinsed off. The most effective way to remove them is to brush them off (Barr, 2003). A piece of cloth can help to remove them.

Finally, fire ant populations can be reduced in sensitive areas by judicious use of insecticidal contact poisons and/or baits. Such areas might include areas around buildings (to reduce risk of ants foraging inside), places where equipment is stored on the ground, and places where people congregate for breaks or to process samples. Control of imported fire ants is beyond the scope of this paper; consult your local Agricultural Extension office for current recommendations and be sure to read, understand, and follow all label instructions on control products.

Actions to Take if Stung

While very rare, a potentially life-threatening systemic reaction to stings can occur in sensitized individuals. This reaction is termed anaphylaxis or anaphylactic shock. All laboratory and field personnel should be required to learn the symptoms associated with anaphylaxis. These are:

- The skin frequently shows symptoms first. Hives, itching, swelling, redness, or a stinging or burning sensation may develop away from the sting.
- The loss of fluid from blood vessels causes a drop in blood pressure and the individual may feel light-headed or even lose consciousness.
- Anaphylaxis can cause obstruction of the nose, mouth, and throat. Individuals may first notice hoarseness or a lump in the throat. If the swelling is very severe, it shuts off the air supply and the individual experiences severe respiratory distress.
- The airways in the lungs can constrict, causing chest tightness, shortness of breath, and wheezing—the classic symptoms of asthma.
- The gastrointestinal tract often reacts, especially if the allergen is something that was swallowed. The person may experience nausea, vomiting, cramping, and diarrhea.
- Women may experience pelvic cramps due to contractions of the uterus (National Jewish Medical and Research Center, 2005).

Numerous Internet sites and Extension fact sheets list symptoms and most include neurological symptoms such as an "impending sense of doom" felt by the victim. Sting victims who voice such concerns should be taken seriously, and medical attention should be sought immediately. Some people are more likely to suffer severe reactions to stings than others; these include multiple-sting victims, people who have suffered stings before, people who have allergies to other insect stings or other severe allergies, and people who are immunosuppressed. It has been recommended that hypersensitive individuals carry an injector kit containing a preloaded epinephrine injector (or syringe) because immunotherapy can be an effective tool to reduce the risk of anaphylaxis (Freeman et al., 1992). People who believe they may be hypersensitive should consult with an allergist (Solley et al., 2002). Fire ant whole-body extract is the only reagent presently available for immunotherapy for fire ant venom allergy (Stafford, 1996). Those who are sensitive to insect stings should probably not begin working with imported fire ants.

Numerous recommendations regarding first aid for fire ant stings can be found in Extension publications and on the World Wide Web. Much of the information is duplicative, and rather than cite numerous sources, this article provides general information (common between sources) that supervisors and workers should be aware of. It should be noted that once a fire ant stings, injecting venom into the skin, pustule formation is not stopped by application of topical steroids, antibiotics, or epinephrine (Parino et al., 1981). For typical reactions to stings (burning sensation, localized reaction, and pustule formation), some reasonable steps can be taken to lessen discomfort and help prevent secondary infection. Alterna-

tively, most people can usually do nothing at all, and the pustules will eventually fade. The majority of available sources have the following suggestions in common:

- Apply a cold compress to the affected area and elevate it.
- Gently wash the affected area with soap and water, taking care not to break the blisters.
- Use over-the-counter sting relief medications if desired.

It is important that sting victims resist the temptation to scratch the affected area, since breaking the blisters or pustules can result in serious secondary infection. Infections can be far more serious than the initial sting and pustule formation.

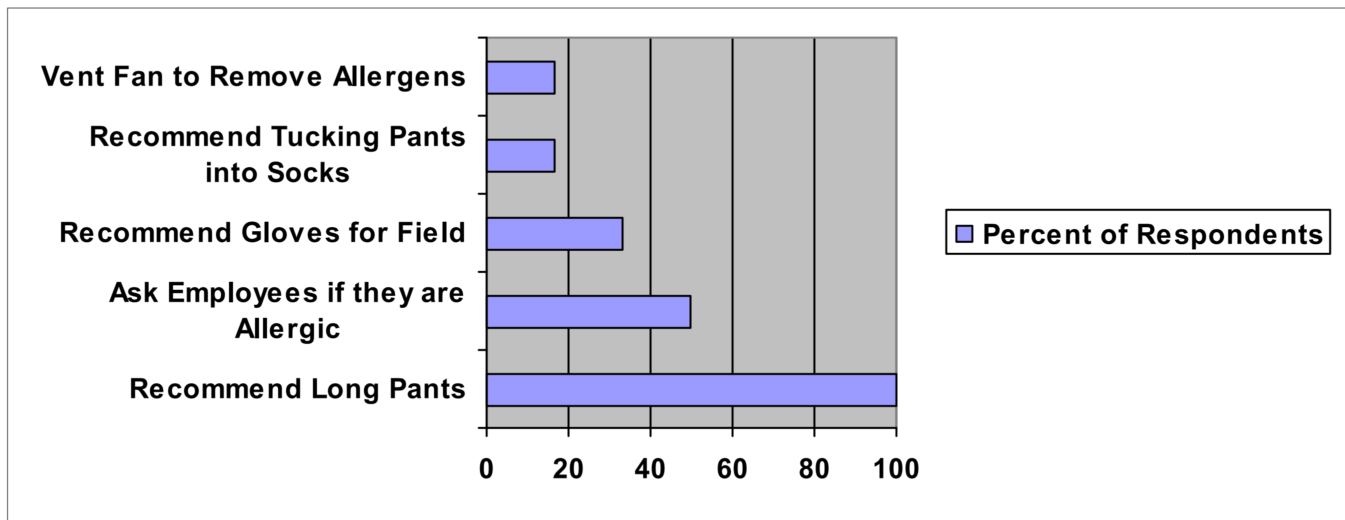
Relatively few people responded (N = 6) to the informal poll distributed by the authors; nonetheless, some interesting information regarding fire ant safety can be gleaned from the responses. None of the respondents had any formal SOPs in place for handling imported fire ants, and no respondents indicated that they required PPE in the laboratory or field, although one indicated that long pants and closed-toe shoes are a requirement in the workplace. Eighty-three percent indicated that they provide gloves in the laboratory and/or field but do not require personnel to wear them; 50% of respondents also provided rubber boots in the field. Additional information from the poll is given in Figure 5. Only one respondent indicated that a vent fan was present in the rearing facility to maintain negative pressure and remove potential allergens; however, the poll's questions were open-ended so it is unclear if other participants may have had similarly equipped facilities. Adequate building ventilation (e.g., BSL-2 laboratory) is recommended for any insect-rearing facility. One respondent indicated that Benadryl® and sting ointments were provided in first aid kits, but epinephrine autoinjectors were not due to legal concerns and the potential consequences of administering medication without the required licensing or training. Providing medication in first aid kits could lead to similar consequences should someone have an adverse reaction to the medication or experience other problems. The general consensus among respondents was that doing nothing (other than avoiding scratching) and allowing the blisters to fade naturally were the best courses of action if stung.

Conclusion

Imported fire ants are a potential hazard to laboratory and field personnel. Suggested PPE and safety precautions may be useful for lessening the incidence or frequency of stings, but there are no foolproof methods for avoiding stings altogether. The recommendations contained herein are provided for information only, with the realization that individuals who have experience with imported fire ants can generally assess their own tolerance for stings and act accordingly. At a minimum, everyone involved in any aspect of fire ant research who risks stings should be familiar with the symptoms of anaphylaxis. Access to a landline or cellular telephone to summon emergency help if necessary would also be helpful. Surprisingly, the only mention of this in the informal poll, other than asking new hires if they are allergic to stings, was made by two respondents who indicated that "additional precautions" would be taken if a worker exhibited sensitivity to stings. New personnel, especially those who are unfamiliar with imported fire ants, should

Figure 5

Results of an informal poll of fire ant researchers indicating the percent of respondents (N = 6) who recommend various safety precautions when working with imported fire ants.



receive appropriate, task-specific training on safety and handling procedures for imported fire ants. Supervisors should be certain that employees understand the risks inherent in working with imported fire ants and encourage them to make informed decisions regarding their risk through consultation with an occupational health professional and/or allergist. Workers should be prepared at all times to deal with potentially life-threatening anaphylaxis and should not hesitate to seek emergency medical care if they or their coworkers experience symptoms. While the vast majority of people have no adverse reaction to stings other than some discomfort and pustule formation, a quick decision to call for help could mean the difference between life and death for an unfortunate few.

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Book Review

Reviewed by Jens H. Kuhn

Harvard Medical School, Southborough, Massachusetts

Viral Haemorrhagic Fevers—Perspectives in Medical Virology (Volume 11)

By Colin R. Howard

Amsterdam, The Netherlands: Elsevier (2005)
205 pp., $130, hardcover
ISBN: 0-444-50660-8

Viral hemorrhagic fevers (VHFs) are severe clinical syndromes of humans, often resulting in extraordinarily high lethality. The diseases are characterized by initial influenza-like symptoms followed by varying degrees of capillaropathy, consumption of blood clotting factors with occasional hemorrhages from mucous membranes, and terminal multiorgan failure. VHF outbreaks are observed in distinct geographical locations consistent with the range of known animal hosts. Outbreaks occur after direct or indirect contact of humans with these animals, which are, in most cases, arthropods or rodents. All known VHF-causing agents can be classified into one of four families: *Arenaviridae*, *Bunyaviridae*, *Flaviviridae*, or *Filoviridae*. Some VHFs, such as the flaviviral diseases yellow fever or dengue fever, have or had tremendous relevance for public health because of tens of thousands of human cases per year. Other VHFs, such as that caused by the ebolaviruses or the arenaviral Lassa fever are of true interest for particular nations only, but have become known to many U.S. households because of Hollywood productions and popular science publications. At the same time, exotic VHFs such as Crimean-Congo hemorrhagic fever, Omsk hemorrhagic fever, Kyasanur forest disease, or Guanarito virus infections are largely unknown even in the scientific community despite their local importance. Depending on the presence or absence of specific antivirals or vaccines and the recorded severity, VHF agents are classified as pathogens requiring facilities certified at BSL-2 (Dengue viruses 1-4, Yellow fever virus), BSL-3 (hantaviruses), or BSL-4 (filoviruses, Crimean-Congo hemorrhagic fever virus, Lassa virus, Machupo virus, and Omsk hemorrhagic fever virus). All these viruses are considered potential agents for biological warfare and terrorism activities and hence are National Institute of Allergy and Infectious Disease Priority Pathogens ranging from Category A through C. The overall boost in research on Priority Pathogens and the increasing number of high-containment laboratories make hemorrhagic fever-causing viruses important for all biosafety and biosecurity professionals.

Colin R. Howard, a well-known scientist with practical experience in the VHF field, recently published *Viral Haemorrhagic Fevers*, a text "aimed at healthcare workers, clinicians, and microbiologists." This handy book contains clearly and well-written self-contained chapters describing each of the four viral families to which VHF agents have been assigned. The different viruses and diseases are treated separately and in detail in the context of history, epidemiology, molecular biology, clinical presentation, diagnosis, treatment, and prevention. Hence, the book will definitely appeal to the intended target audiences.

However, reading this volume left me with mixed feelings. On the one hand, the introductory and commentary chapters at the beginning and end of the book are worthy reviews themselves, and are wonderfully written and scientifically sound. Furthermore, fascinating historical subchapters for most viruses are filled with anecdotes of research pioneers and ancient observations, all of which raised my curiosity. Especially the subchapters on yellow fever virus and dengue viruses 1-4 are recommended to the reader. The book is surprisingly current—even recently discovered exotic agents such as Alkhurma virus or Garissa virus are briefly mentioned. On the other hand, the epidemiological subchapters are sometimes incomplete or flawed. For instance, while one could not expect the very recent marburgvirus outbreak in Angola to be mentioned, Howard should have mentioned the successive outbreaks from 1998-2000. An ebolavirus fever outbreak in Sudan is mistakenly placed in 1996, when in fact it occurred in 2000; a marburgvirus fever outbreak that allegedly occurred in 1982 has never been proven; and ebolaviruses were discovered in 1976, not in 1972. Worse, the viral taxonomy used in the book is not current and Howard randomly switches between virus and disease names and italic or nonitalic typing. The author assigns "influenza" to the family "Myxoviridae" (should read influenzavirus and *Orthomyxoviridae*), discusses "Congo-Crimean haemorrhagic fever" (should read Crimean-Congo hemorrhagic fever virus), or states that the "various strains and isolates of LCM [lymphocytic choriomeningitis] are now considered to be a genus within the family Arenaviridae"—when in fact *Arenaviridae*

contains only one genus (*Arenavirus*), which in turn harbors many different species with LCMV being one of them.

The book is also poorly edited. Among the harmless typographical or grammatical mistakes are countless examples of wrongly spelled scientific vocabulary: "*Dunacentor*" ticks (*Dermacentor*), "Thottopalyan virus" (Thottopalayam virus), "haemophagic fevers" (hemorrhagic fevers), "Guillaime-Barre syndrome" (Guillain-Barré syndrome), "aenavirus" (arenavirus), or "fucin" protease (furin) are just some examples. The described diseases are most often referred to in a "colloquial" manner—mentioning the latest WHO International Classification of Diseases (ICD-10) designations would have been helpful. Tables in the book are sometimes difficult to read or incomplete, and they are randomly placed; one reference to a table forced me to go back 16 pages to find it.

Viral Haemorrhagic Fevers contains too few references for the specialist or the interested outsider, and the bibliography is heavily biased towards literature written in English and published in Western journals. This is not a trivial point. Omsk hemorrhagic fever, for example, gets cursory treatment with little or no reference to several hundred Soviet/Russian references on the subject.

It is also unclear why the author included hantavirus pulmonary syndrome in the book—a disease that is not characterized by hemorrhages. Likewise, lymphocytic choriomeningitis is described but there is no mention that hemorrhagic manifestations have actually been recorded in rare cases. Furthermore, the text occasionally contradicts itself. While tables list Whitewater Arroyo virus as not being associated with human disease, the accompanying text states that several severe cases of VHF caused by this agent have been recorded.

Viral Haemorrhagic Fevers has the potential to be a wonderful and educational textbook for students and professionals alike. However, a new, carefully edited, and better-referenced version should be prepared before the book could be truly recommended to a wider audience.

Ask the Experts

John H. Keene

Biohaztec Associates, Midlothian, Virginia

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

Non-Compliant Biocontainment Facilities and Associated Liability

It is a fact that a large number of biocontainment laboratories in universities, corporations, and other institutions are not up to current standards as stated in the 4th edition of the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) and that a significant number of new biocontainment laboratories are being built. Because of the public perception with regard to the safety of these facilities, a number of institutions and biosafety professionals are concerned when, for one reason or another, the institution does not follow the guidelines for insuring containment. Several questions have been raised concerning liability for failure to follow the guidelines.

For an expert answer to these questions, I asked R. Leonard Vance, JD, PhD, PE, CIH, Associate Professor, Department of Epidemiology & Community Health, Medical College of Virginia, Virginia Commonwealth University to respond. Dr. Vance is the former Director of Health Standards for Federal OSHA under the Reagan Administration. His answers follow:

What is the potential liability, in the event of an injury, for an institution that does not follow the BMBL or the NIH Guidelines for the Design and Construction of Laboratories (NIH-DCL)?

Assuming:

1. The BMBL is the "standard of the industry" when it comes to how biocontainment laboratories/facilities should be operated;
2. The BMBL states that biocontainment laboratories should be validated with regard to design construction and procedure prior to initiating work; and

3. The NIH has developed a set of design and construction guidelines that also have been accepted as the "standards of the industry."

The question raised has several possible responses and encompasses issues of common law negligence, contractual liability, and regulatory compliance. Let us begin by assuming all three of the conditions above are true and the laboratory we are discussing operates outside of either set of guidelines. What are the consequences for the following entities?

- A person not employed by the lab who is injured by an incident occurring in the lab;
- The owner of the lab;
- A consultant who designed the lab;
- An employee working in the lab who is injured.

Negligence and Standards of Care

The elements of common law negligence are duty, breach of duty causation and damages. Duty is established based on the relationship, or absence thereof, between the parties involved in an injury. Breach of duty involves the question of determination of the standard of conduct to which the defendant must conform. It is that standard one speaks of when discussing the standards of care. The standards of care depend on facts surrounding injury to the plaintiff and the relationship of the parties. Ordinarily, the standards of care are those which a prudent person would use under like circumstances. The legal question to be answered by the court is: Was the defendant's conduct reasonable in light of the apparent risk?

Health and safety standards issued by safety organizations such as the National Safety Council, The American Conference of Governmental Industrial Hygienist (ACGIH, The American National Standards Institute (ANSI) have been routinely used to establish the standards of care in negligence litigation. The BMBL and NIH-DCL and other similar federal guidelines are simply another such set of guidelines counsel for an injured person would introduce in connection with establishment of standards of care. If the guidelines had not been followed, an injury victim would likely introduce them for the purpose of showing noncompliance with the standards of care. If the guidelines had been followed, a defendant, e.g., the owner of the lab or its designer, would likely introduce them as defense evidence. When such guidelines are used as evidence, the evidence must ordinarily be presented by expert witnesses. Thus, either a plaintiff or a defendant might retain a biosafety professional to testify as to the standards of care.

Suppose a person is injured because of an incident in a laboratory. The injury is caused (arguably) by failure of the lab personnel or owner complying with the guidelines. Who is liable for the damages incurred as a result of the injury? The owner of the lab obviously is exposed to liability in a case like this. If the injured person is an employee of the lab, the victim's sole recourse is workers compensation. For anyone else, recovery would be through a suit against the owner of the lab. A design professional who designed the lab would be exposed to liability, as well.

Contractual Liability could also result from noncompliance with the guidelines. Conditions in a grant, e.g., NIH, Department of Defense, Department of Energy, U.S. Department of Agriculture, etc., establish the requirements applicable to the parties to the contract. Failure to perform in conformance with the grant could trigger an order to return all funds expended under the grant. Thus, an institution could find itself obligated to return grant funds already expended if found in noncompliance with the grant requirements.

Regulatory Liability also exists when the guidelines have been incorporated into regulations. Consensus standards are often adopted as regulations by governmental agencies. OSHA has adopted ANSI and ACGIH consensus standards, as well as CDC Guidelines, as Agency standards. In the case of the BMBL, there is a requirement that guidelines be followed as a prerequisite for working with Select Agents under the Public Health Security and Bioterrorism Preparedness and Response Act of 2002. This then makes the status of the guidelines in establishing the standards of care much more firm.

Who is ultimately responsible for insuring the containment facility, which belongs to the institution, is in compliance, both from a physical construction standpoint and from an operation/management standpoint?

Each institution is ultimately responsible for insuring that a containment facility belonging to the institution conforms to the standards of care. This is a common law duty recognized throughout the United States. Many institutions may be shielded from liability by the doctrine of sovereign immunity. How sovereign immunity applies varies from jurisdiction to jurisdiction, but sovereign immunity is a defense to a claim of liability. However, sovereign immunity does not lessen the degree of obligation an institution has to operate within the applicable standards of care, both from a physical construction standpoint and from an operational/management standpoint. It simply prevents a claimant from recovering money damages from the institution.

Could the Chief Executive Officer also be found liable if he or she knew about the failure to comply, and/or could the head of engineering or of health and safety

also be implicated if they failed to follow the requirements and did not provide appropriate guidance and/or warning of failure to comply?

Yes, given that the injury is shown to be a result of a specific breach of duty set forth by the applicable standards of care. This is similar to the situation of a malpractice claimant who files suit against both the hospital and the physicians involved with his treatment. Individual officers, or employees of an institution, may be found liable for failure to insure compliance with the applicable standards. Even in the case of a governmental institution, individual officers or employees of the institution may be found liable in their personal capacity, while the institution itself is shielded from liability by the doctrine of sovereign immunity.

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 this "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.

Mumps Outbreak Update Information

Changes in recommendations have been made for determining HCW immune status due to the 2006 outbreak published. Determining the immune status of personnel; either by documentation of two MMRs, a positive mumps IgG or history of physician-diagnosed mumps, or birth before 1957, is recommended.

For information regarding clinical disease, infection control measures, and updates on vaccinations see: Mumps–Technical Q&As for healthcare professionals at www.cdc.gov/nip/diseases/mumps/mumps-tech-faqs.htm#exp.

Additional information may also be found at Mumps information for healthcare professionals.

www.cdc.gov/nip/diseases/mumps/default.htm

CDC Health Update: Inhalation Anthrax Case Investigation, Pennsylvania, New York City–Update, 2/24/2006

The recent case of inhalation anthrax presented investigators with some interesting challenges. Was this a case of bioterrorism or something else? The following link provides an update to this somewhat unusual, but not totally unexpected exposure.

www.bt.cdc.gov/agent/anthrax/han022406.asp

Avian Influenza Virus

Avian influenza virus usually refers to influenza A viruses found chiefly in birds, but infections can occur in humans. The risk is generally low to most people, because the viruses do not usually infect humans. However, confirmed cases of human infection have been reported since 1997.

The CDC updated the following web site on April 24, 2006, with current information regarding the status of this growing concern.

www.cdc.gov/flu/avian/

Emerging Infectious Diseases (EID)

The May 2006 issue of the EID is dedicated to the reemergence of Tuberculosis. This disease has shown extreme resilience during the past century in spite of the advancements in therapy. For more information, visit www.cdc.gov/ncidod/EID/index.htm

Approaches to Safe Nanotechnology: An Information Exchange with NIOSH

Nanotechnology–do you know what it is? Will your industry be impacted by it? What are the occupational health and safety issues? For an introduction to Nanotechnology, check out the following link. The document is still underdevelopment but provides an entrée to this fascinating field.

www.cdc.gov/niosh/topics/nanotech/nano_exchange.html

New ABSA Members for 2006

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Calendar of Events

August 28-29, 2006
American Biological Safety Association (ABSA) Summer Seminar Series
Doubletree Guest Suites, Durham, North Carolina
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

October 15-18, 2006
American Biological Safety Association (ABSA) 49th Annual Conference
Marriott Copley Hotel, Boston, Massachusetts
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

October 7-10, 2007
American Biological Safety Association (ABSA) 50th Annual Conference
Opryland Hotel, Nashville, Tennessee
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

October 19-22, 2008
American Biological Safety Association (ABSA) 51st Annual Conference
John Ascuaga's Nugget, Reno, Nevada
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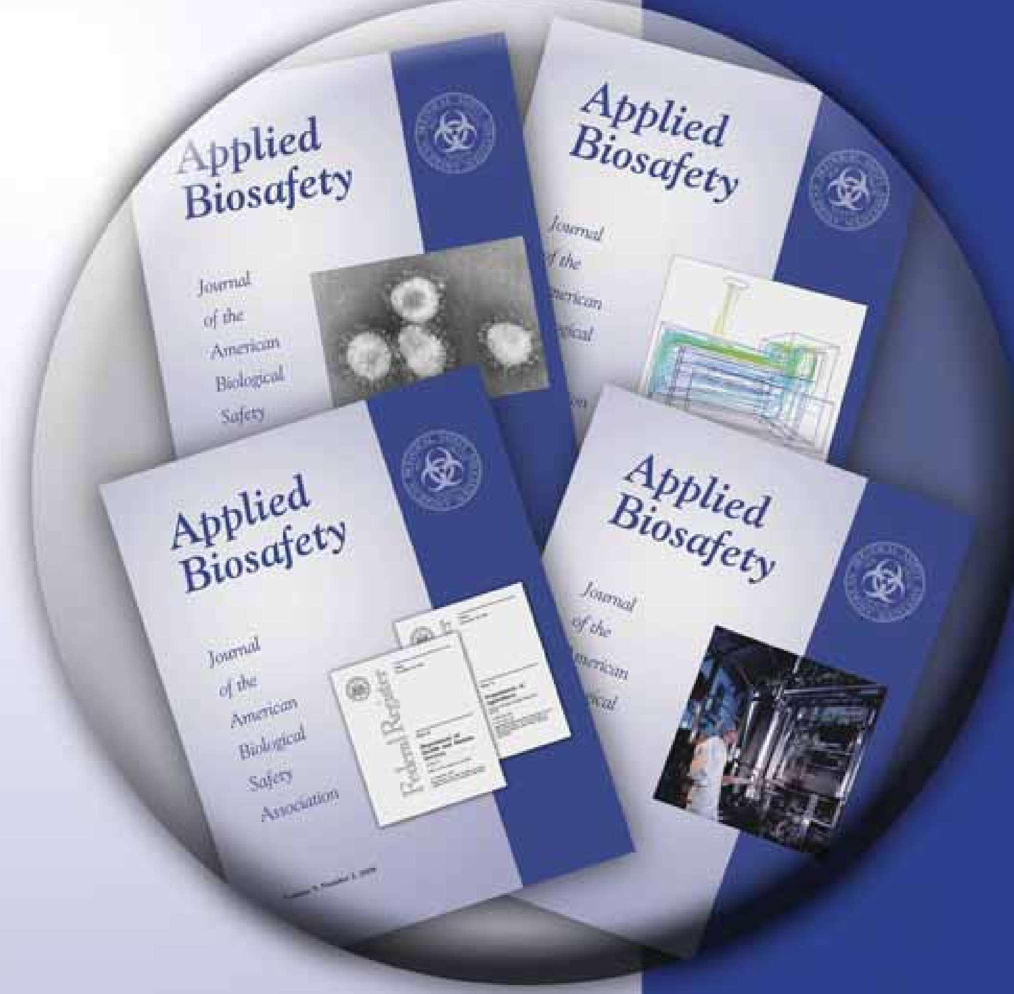

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A free preview of *Applied Biosafety,* Volume 10, Number 1, is at www.absa.org/resabj.html; for userID, type in “abj273” with password “8f9T74”

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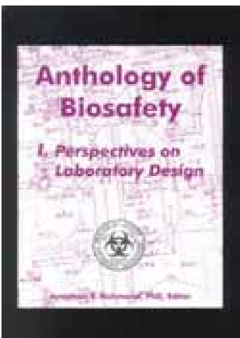

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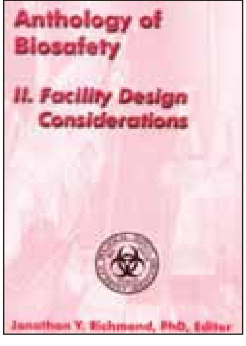

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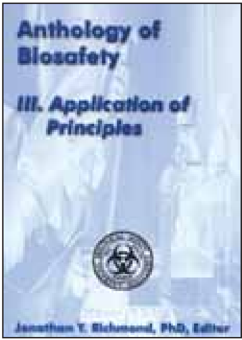

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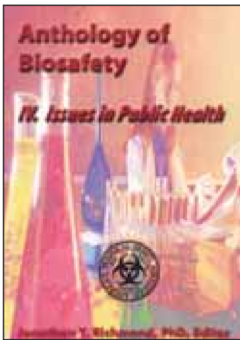

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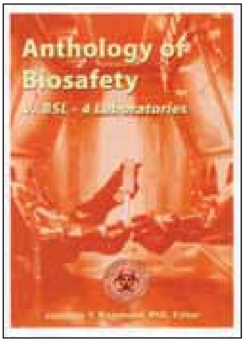

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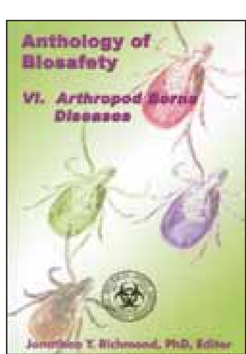

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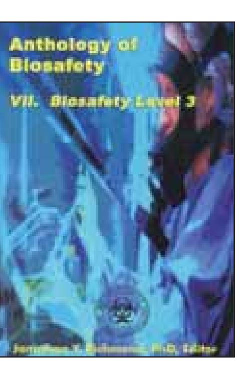

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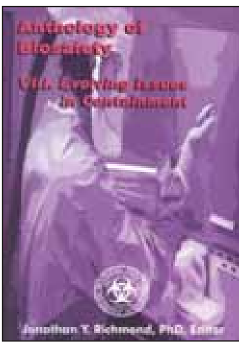

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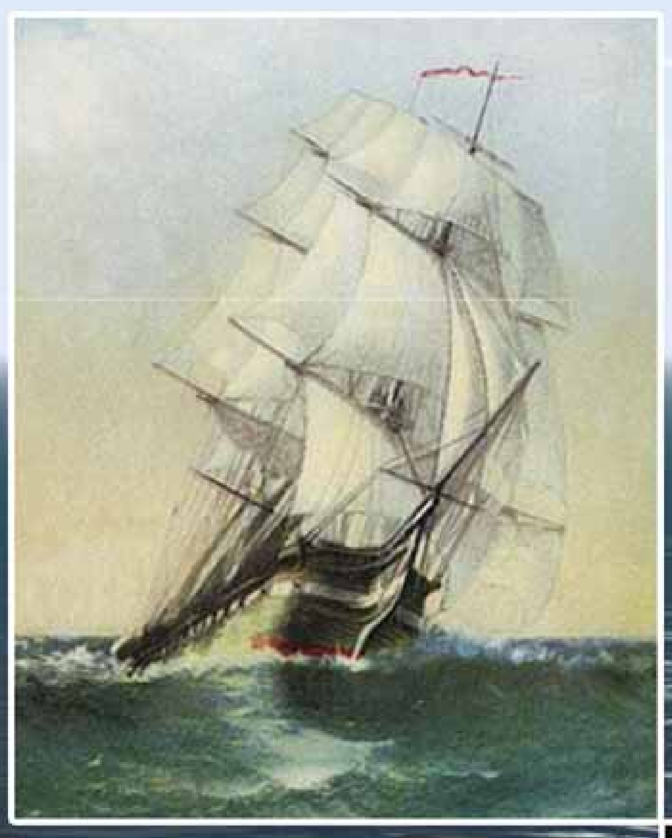

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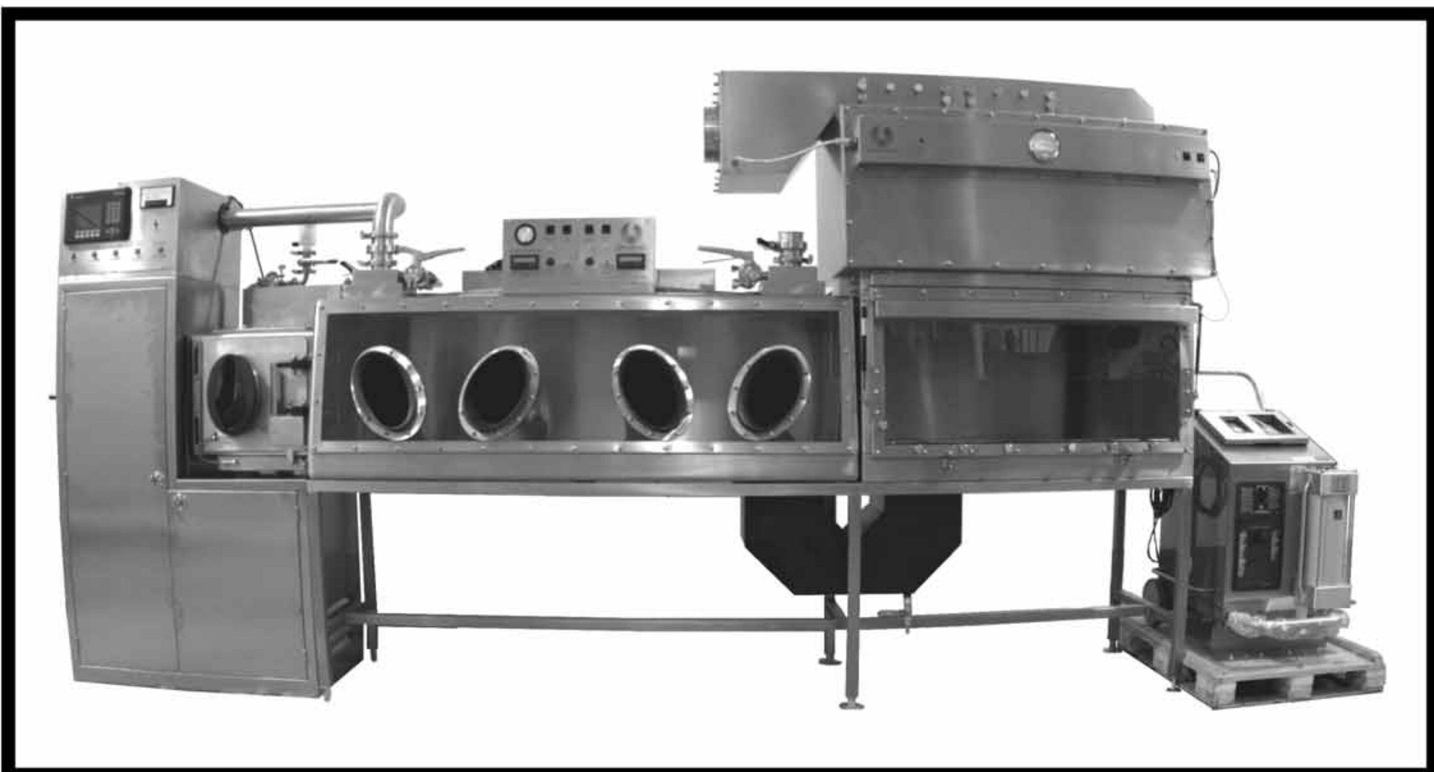

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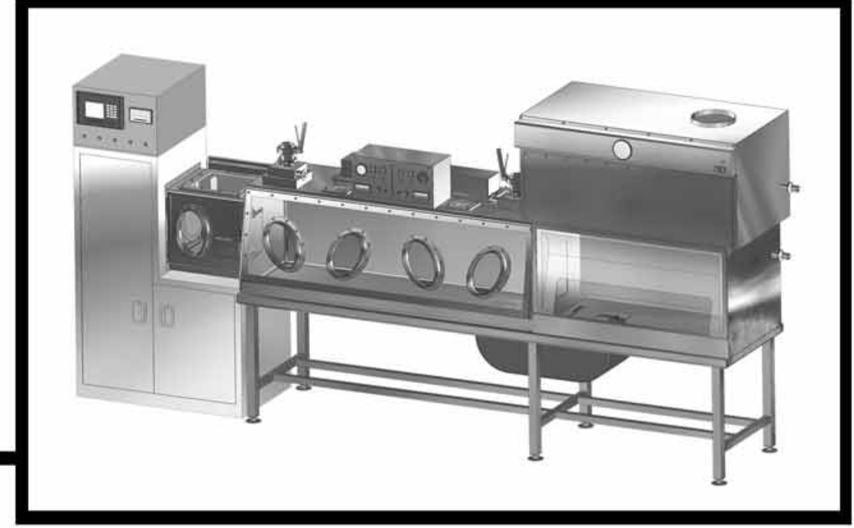

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