Safety by Design:
2015 Biosafety Monograph

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Contents

Foreword

The life sciences community has a long and honorable legacy in the safe conduct of research involving hazards presented by microorganisms. This legacy of biosafety reaches back to the origins of modern microbiology in the late 19th century, and is more concretely documented in publications by leaders in the field such as Sulkin and Pike, and Arnold Wedum starting in the mid-20th century. In 1978, the NIH published the *Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Research*," prepared by the Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts. The *Monograph* is an incredibly detailed, 230-plus page document that is a treasure trove of what to do and how to do it, and how to do it safely in the lab. I would assert that this guidance has stood the test of time. It is also the document around which the NIH organized a Safety by Design Symposium in April 2011.

I think it is critically important as we go forward with biological research that we find avenues to educate practitioners and the public about biosafety in the broadest sense. Biosafety is the foundational discipline for what has been termed more recently ˙bio risk management". The European community has taken this broader approach, as evidenced in the 2008 CEN (European Committee for Standardization) Workshop 31 - Laboratory Biosafety and Biosecurity. In the US, we've focused on emphasizing, in addition to classical elements of biosafety, biosecurity, personnel reliability and environmental impacts. With respect to biosecurity (which has many definitions) and personnel reliability, biosafety clearly includes the critical elements of access controls, training, risk assessment, and dedicated, knowledgeable laboratory oversight.

Moving into the future, what are the high-risk pathogens, and high-risk activities? There are and will be more genetically engineered organisms, engineered cells and applications involving recombinant

DNA. Synthetic biology has been the subject of several of its own conferences, discussions, and guidance, and raises numerous issues related to biosafety and biosecurity. Nano-technology is finding applications everywhere, including in medicine and the life sciences. The properties of some of these nano-materials have the potential for significant biological impacts. How are we going to work with the communities of bioengineers and materials scientists, which are largely composed of individuals with no microbiological training or foundation in safety fundamentals? They probably need a lot of education and hand-holding to be able to work with these materials safely, and conversely, the biosafety community may need to broaden its own technical understanding in order to develop sound, scientifically based guidelines for managing the biological risks of new materials and processes.

And last, but definitely not least, we surely will be confronted with emerging pathogens for which we need to develop appropriate biosafety guidelines and risk management approaches. But we face a real communications challenge because the folks who are not scientists – our elected officials, our friends, our neighbors, even our families – are afraid of the microorganisms and diseases that we work with, and they're not familiar with how we manage the risks. Biosafety professionals have a golden opportunity to take a leadership position in "safety by design" as we face a future of an increasing number of challenges, decreasing tolerance for risk, and increasing demand for concrete assurances that the scientific community is acting responsibly.

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The findings and conclusions presented here are those of the author and do not necessarily represent the views of the Department of Health and Human Services or its components.

Preface

Significant advancements in the discipline of biosafety and the practice of safe science have occurred since the National Cancer Institute published the *Laboratory Safety Monograph* in 1978. To examine these advancements, the National Institutes of Health, Office of Research Services, Division of Occupational Health and Safety, sponsored a Safety by Design Symposium in 2011 titled "A 33-year Legacy: The NIH *Laboratory Safety Monograph* Revisited." Roughly 200 biosafety experts, environmental health and safety professionals, scientists, laboratory technicians and managers, architects, and engineers from academia, private industry, and the federal government attended the three-day event. Through formal presentations and in work group settings, these skilled and knowledgeable professionals considered issues affecting both biosafety and scientific progress emerging over the past three decades such as responsible research and risk assessment. They studied the progressions in biomedical research involving select agents and other high-risk pathogens, and new technical developments. They assessed and often questioned what constitutes good laboratory practice, the proper selection and use of containment equipment, the most efficient and safe design and operation of research facilities, and effective management.

The information vetted at the Safety by Design Symposium was the source for the guidance and recommendations presented in this *Safety By Design: 2015 Biosafety Monograph*. The information is authoritative and reflects the most current scientific reasoning, research and experiences of some of our nation's leading experts in the diverse disciplines that support and enable biomedical research. The information is advisory in nature and does not set mandatory requirements. It is meant to help advance a culture of research safety and responsibility in the conduct of biomedical research involving select agents and other high-risk pathogens. Its purpose is to encourage principal investigators, safety

professionals, and institutional officials to seek new and improved methods of biohazard control, and to apply professional judgment in the selection and exercise of laboratory safety practices and principles.

Major sections of this *Biosafety Monograph* address the oversight and support of biomedical research including roles and responsibilities, emergency response procedures, medical support services, and training. A section on laboratory practices deals with laboratory techniques for biohazard control, the care and use of laboratory animals, necropsy, and decontamination and disposal. A section on containment equipment provides information on the state-of-the-art design and proper use of biological safety cabinets. A section on special laboratory design addresses design and operation requirements and considerations applicable to Biosafety Levels 3 and 4 laboratory and animal facilities. Two sections address responsible research and risk assessment and are presented here as they were at the Symposium. These sections reflect the wisdom, work, and expertise of the authors.

Preparation of this *Biosafety Monograph* was truly a team endeavor. I thank our many scientific and professional colleagues who generously gave their time and brought wide-ranging expertise to the roles of lead writers, technical reviewers, and subject matter advisors. I also thank those who provided superb, instructive presentations at the Safety by Design Symposium, those who courageously filled the role of work group moderators, and the many Symposium attendees who contributed to the lively exchanges that occurred throughout the meeting. Each idea presented, question raised, discussion, and debate – whether occurring during the formal program, in a work group, or over coffee or lunch – contributed to the collective thinking, scientific understanding, and guidance presented in this *Biosafety Monograph*.

Lastly, I thank W. Emmett Barkley for serving as Co-editor and for leading the development of this *Biosafety Monograph*. As the originator and principal writer of the 1978 *Laboratory Safety Monograph*, Dr. Barkley brought all-important experience and knowledge to the creation of this *Biosafety Monograph*. I also thank Dr. Barkley for his vision for a Safety by Design Symposium and his tenacity, toil and leadership to make it happen.

Moving forward, I hope each of us who work to carry out or support research programs involving potentially hazardous organisms will consider safety by design a key principle in all that we do. From the design and construction phase through the initial risk assessment and conduct of research protocols to the final disinfection and disposal of hazardous wastes, designing established biosafety principles and practices into every aspect of our programs will ensure an environment where safe and responsible research can thrive.

As we work together "to seek fundamental knowledge about the nature and behavior of living systems and the application of that knowledge to enhance health, lengthen life, and reduce illness and disability,"[1] I hope the guidance provided in this *Safety by Design: 2015 Biosafety Monograph* will contribute mightily to our shared pursuit to create and preserve a safe research environment.

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[1] NIH Mission Statement

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A Culture of Safe Science

Responsible Research in the Biological Sciences

By way of introduction, it is important to emphasize that more than 100 years of research has been done on many species of pathogenic bacteria, viruses, and parasites and essentially n=1, with respect to bioterrorist activity, namely the anthrax episode of 2001.

There have been few instances, if any, of documented theft of infectious agents from a university or government laboratory. Some unfortunate episodes have been proven to have derived from laboratory workers forgetting they had autoclaved cultures that were reported to have been lost or missing. The life sciences in the United States have been remarkable in research productivity. Thus, there is serious concern that the United States remains a leader in life sciences research. We must not impede progress with burdensome, unnecessary regulation. That is not to say that regulations are not needed, but regulations should support, not impede, research that leads to discovery and application of achievements in the life sciences to medicine and public health.

"We should be able to provide excellent research and laboratory safety, meeting all requirements, and continuing to recognize the importance of research on pathogens being done with creativity and fostering discovery while maintaining safety and security for all personnel."

– Rita R. Colwell, Ph.D.

One of the difficulties peculiar to current times is that many molecular biologists and biochemists working in laboratories today have never had a formal introductory course in microbiology. It is important

to be trained in methods for transferring cultures, notably pathogens. Aseptic transfer of cultures in the laboratory, avoiding aerosols, is a simple, but important technique. It may be useful to develop a universal introductory short course, perhaps online with a website, on basic microbiology laboratory methods, including laboratory safety. Because of the anthrax episode, there is a concern that cultures of pathogens may be taken from a laboratory for adverse purposes, i.e., terrorism or criminal design. And there is the problem that individuals with access to pathogens may not be sufficiently trained to handle or use them properly. These are the issues we must deal with that are both real and critical.

In the report *Responsible Research with Biological Select Agents and Toxins*, prepared by the National Academy of Science (NAS) committee, physical security, personnel, and issues related to individuals working with select agents, toxins, and pathogens, in general, were considered. The focus, however, was on security, not safety *per se*. The focus in this piece is on individual responsibility and safety. High-containment laboratories (BSL-4) and their operations were not an explicit focus of our report, although select agent research is done in such laboratories. Nevertheless, the report covered handling pathogens regardless of biological safety level of the laboratory facility.

Approximately 82 agents or toxins have been placed on the select agent list, with other additions proposed. The NAS report strongly emphasized that the select agent list should be reviewed. That is the select agent list should be prioritized, with levels of risk assigned, that is, categories of very dangerous, moderate, or routinely studied pathogens handled safely in a BSL-2 laboratory. Great care must be taken when addition to the select agent list is considered. A proposal was made to add SARS and H1N1 viruses to the select agent list. The question then arose if all such pathogens are added to the select agent list, how can research proceed with the burdensome procedures and regulations that must, by law, be followed for the most dangerous pathogens if those procedures and regulations are applied to all pathogens? The select agent list must assign categories that address safety and caution but allow research to proceed without severe impediment to scientific research and development.

The preparatory work of the NAS committee for its report yielded findings complementary to those of many earlier studies, e.g., Executive Order Working Group Report, National Science Advisory Board for Biosecurity Report, Defense Science Board report, and others.

The committee preparing the NAS report comprised representatives from industry and academia, the non-profit sector, a former FBI agent, and a psychologist who had done extensive personality analyses of laboratory researchers. The committee came to full consensus on recommendations of the report, with no minority report.

Some important principles derived from the committee's work presented in the report are as follows.

Research on biological select agents and toxins is essential to the national interest of the United States. There are concerns associated with select agents, but any select agent program must focus on those agents of potential use as bio-threat agents, not pathogens on which studies are done to prevent disease and provide public health protection.

It is important to emphasize that misuse of biological materials is taboo within the scientific community of our country. An environment where full understanding of how to work with these materials is critical for every laboratory. It is the responsibility of every principal investigator to mentor staff, students, and trainees, inculcating an attitude of responsibility, and concern for safety, not only for the individual, but for all personnel in the laboratory with whom the individual works.

A strong theme of the report and of my message here is that research is a critical element of the well-being and security of our nation. We must foster a culture of trust and responsibility in all laboratories where work is done with microorganisms, especially those known to be pathogenic.

Other issues covered in the report concern problems that have arisen from oppressive regulation, that is, excessive demands made of those who have been working with select agents. We learned of instances where investigators gave up trying to deal with the demands of regulations and requirements for working with microorganisms on the select agent list and autoclaved their culture collection accumulated during years of research, including cultures inherited from earlier researchers. This represents a tragic loss of scientific material and the

wealth of experience that can never be replicated or replicated at great expense. It is a sad outcome for our country.

We learned of young scientists who refused to deal with the burden of regulations, reports, and constant inspections that are associated with an assumption or implication of wrong doing, an attitude of some inspectors investigating laboratories working with highly pathogenic agents, namely that malevolent work was being done. This loss of talent must be taken into account when regulations and requirements are proposed. The point is that balance is necessary, with respect to procedures and requirements, despite good intention to ensure protection, with the result going awry. It is critical that the mechanisms of infectious agents be studied so that vaccines can be developed and preventive measures against pathogens established, laudable goals that come from research.

A recommendation was made to establish a biological select agent and toxins advisory committee that included scientific researchers, laboratory directors, and experts in biosecurity, animal care and use, and compliance. A select agent advisory committee can provide information and advice on select agent listings. There was concern about a congressional mandate to add additional pathogens to the select agent list, an action that would inhibit research in those laboratories in the United States currently carrying out research on proposed additions to the list.

Harmonization of regulatory policies and practices is another consideration that is needed. Different agencies have different requirements. An example that perhaps may be extreme, but makes the point, is as follows. Inspections of a BSL-4 laboratory were done by different agencies, as required by law. The inspector of one agency reported that a window must be installed in the laboratory. The other agency inspector reported that a window in the laboratory must not be installed. Literally, the select agent researchers were caught in a "Catch 22", no doubt an extreme situation, but it actually occurred.

In any case, harmonization of rules and regulations of the agencies doing oversight and review is clearly needed. Key constituency groups need to be involved and that means researchers as well as the laboratory managers in the agencies. The select agent program needs to be updated

to balance security with the ability to carry out research productively and effectively.

Another aspect to consider is screening individuals who work with select agents. Current screening practices that are done by the FBI are sufficient, but there is concern about some of the disqualifiers that are automatic and permanent. That is, databases used in screening must be consistent with government practices and adequate to assess whether an applicant possesses the appropriate background and the FBI background check does do this effectively. But there is no "silver bullet" in the form of a personal characteristic or individual feature to screen out those individuals who might turn to terrorist activity or have an underlying psychological desire to do so. Thus, an appeal process is needed to provide the opportunity to consider circumstances that might otherwise disqualify an individual from doing research on a select agent. At the present time, an indiscretion at the age of 15, a transgression, and there are many that come to mind, will eliminate the candidate, who may now be 45 years old, a responsible citizen and an effective researcher for many years. The single transgression as a teenager would prevent him or her from working with a select agent. Thus, an appeal process is needed to avoid eliminating from the talent pool some very talented scientists who could make very important contributions to the body of knowledge on pathogenic microorganisms and the diseases they cause.

Above all, those who operate laboratories, especially those laboratories with student workers, postdoctoral fellows, and trainees, need to make it their goal to inculcate trust and responsibility. Those of us who are, or have been, principal investigators spend time building an *esprit de corps* in our laboratories. We train our students, spend time talking with our students, understand their aspirations, guide their careers, and discuss their research with them. We build trust, loyalty, and compassion.

That trust and inter-personal connection is critical, especially when the mentee has a personal problem or may behave strangely in the laboratory because of personal stress. If a student feels sufficiently comfortable to approach his or her mentor to suggest a fellow student or colleague needs help, that is characteristic of most, if not all laboratories in the United States…or should be. Occasionally an irascible individual may be a laboratory supervisor. If that is the case, it would be useful to

have an ombudsman on the campus, or in the nonprofit laboratory or government agency as a neutral party who maintains both discretion and confidentiality. If an untoward situation arises, the ombudsman could be approached.

Training in scientific ethics and in the potential of dual use research is an important consideration. Most universities have established courses in scientific ethics, good laboratory practices, and the community responsibility of life science researchers.

From the site visits the committee made, it was concluded that federal agencies and professional societies, e.g., American Society for Microbiology, Federation of American Societies for Experimental Biology (FASEB), American Chemical Society and others, should provide education and training, perhaps in the form of workshops on good laboratory practices, bioethics, and topics relevant to select agent research at their annual meetings.

Clearly, as stated above, stratification of the select agent and toxins list is a priority. Stratification on the basis of potential as a bio-threat agent is critical. Also, removal of select agents or toxins from the list, if warranted, should be possible since some pathogens now on the select agent list, based on the historical record of good practices currently followed in the laboratory, do not merit being on the list.

Stakeholders – scientists, working in the laboratory, laboratory managers, those who have a responsibility for a large team of researchers carrying out research – need to be involved in discussion of select agent practices, rules, and regulations, along with agency representatives and those responsible for oversight of laboratory practices. These stakeholders must be involved.

Regulations and requirements must be harmonized. Inspections are not necessarily uniform in interpretation of the rules and requirements. For that reason, inspectors of select agent laboratories should have scientific training. They must, at minimum, understand the science being done and interaction with the laboratories they inspect should be within a harmonized framework, rather than strict adherence without understanding the science being done. Common sense should allow for untoward activity to be stopped or modified so that the research can continue with proper safety procedures in place and followed accordingly. Training for laboratory inspectors is critical because those

doing inspections play a significant role in ensuring laboratory safety. A scientific background and laboratory experience are important criteria for selecting individuals who are responsible for inspecting BSL-2, -3, and -4 laboratories.

Nuclear and chemical agents differ from biological agents, obviously, because biological agents replicate. Counting laboratory test tubes and vials is not an effective or efficient procedure for maintaining records of laboratory cultures. At worst, it offers a false sense of security. Records of which agents are in the laboratory, where they are stored, who has access to them, when access has occurred, and the intended use are the type of information that is valuable and should be maintained. Counting test tubes and counting Petri dishes is highly ineffective and wastes time, at minimum. Test tubes, Petri dishes, and/or flask counting should be required only when transferring materials from one geographical location to another, e.g., moving cultures from a laboratory in Maryland to a laboratory in California, or from laboratory to laboratory within a city or state. The number of vials and plates then needs to be recorded, but within an individual laboratory, record keeping with log entry and log exit for select agent material is most effective to account for and carry out responsible research.

The requirement for safe laboratory practices for security and compliance does involve cost. There should be a separate budget category for these costs. To clarify, rather than having the principal investigator allocate cost of safety requirements for select agents in the research budget, there should be a budget set aside at the agencies, e.g., NIH, NSF, FDA, DoD, for security practices. Such a system is maintained at the National Science Foundation for operation of large equipment, i.e., a telescope, cyclotron or synchrotron. A separate major research facility budget is allocated but is separate from the research budget. This practice should be followed for select agent research laboratories.

In summary, scientific research in the United States, notably research on microorganisms of public health concern and pathogenic microorganisms in general is unparalleled. In developing safety practices, we should not inhibit that research. We should be able to provide excellent research and laboratory safety, meeting all

requirements, and continuing to recognize the importance of research on pathogens being done with creativity and fostering discovery while maintaining safety and security for all personnel.

Reflection on the success of the Asilomar conference that was held years ago may be useful. Scientists at that conference addressed issues of recombinant DNA laboratory safety and proposed a mechanism for ensuring safety and responsibility. Now may be the time to organize another Asilomar type of conference for researchers, laboratory professionals trained in laboratory safety practices, and representatives of agencies to address issues associated with select agent research.

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Supporting Safe Science

Roles and Responsibilities of the Institution, Biosafety Officer and Responsible Official

As repeatedly commented upon during the Symposium, the approach, tone and content of the 1978 *Laboratory Safety Monograph* remain remarkably relevant today, a reflection of the care and seriousness with which the scientific community was considering and integrating advancements in molecular biology and genetics into the conduct of research during that period. The *Monograph's* description of the roles and responsibilities of the institution and biosafety officer are no exception to this relevance.

Then, as now, it was noted that the institution had an obligation to ensure that research was conducted in a manner that recognized and mitigated the risks to the individuals who conducted the research as well as to these individuals' communities and environment. While the *Monograph* makes many specific recommendations for how an institution should meet this obligation, it also appropriately conveys that success depends more upon an institution developing and implementing procedures and processes that fit its culture, integrate its scientists and staff into the process, and allow for sharing of information within and between the institution and other stakeholder institutions and agencies.

> *"The principal qualifications, roles and responsibilities for the biosafety professional envisioned and articulated first in the Lab Safety Monograph are as relevant today as they were when first articulated and have been echoed by fellow scientists globally since first issued."*
>
> – Joseph A. Kanabrocki, Ph.D., CBSP

The role of the biosafety professional circa 1978, as described by the *Monograph*, was already recognized as broad in scope, complex, and essential, but individuals who had the requisite expertise and experience to meet all assigned and assumed responsibilities were deemed rare. In the intervening three decades, the field of biosafety has experienced necessary and continual growth and has gained widespread recognition for its importance to the conduct of safe science. Also during these intervening years, the biosafety professional has been called upon to foray into new areas tangential to, but best conducted in accordance with the same principles as biosafety – namely biosecurity, biosurety and regulatory compliance. Along the way, many other agencies and groups have weighed in on and helped shape – through regulations, guidelines, recommendations, education, and credentialing programs – the role of the biosafety professional.

The federal government, through, for example, NIH *Guidelines*[2], OSHA Bloodborne Pathogens Standard[3], and Select Agent regulations[4] assigns specific responsibilities to effected institutions for ensuring personnel and environmental safety, including responsibilities that are explicitly or implicitly assigned to a biosafety professional. The "bible" of biosafety, the BMBL[5], now in its fifth edition, stresses the importance of risk assessment and the need to have the biosafety professional play a lead role in this responsibility. This is echoed by the NRC *Guide for the Care and Use of Laboratory Animals*[6], which looks to the biosafety professional to advise on occupational health and occupational hazards.

As the role of the biosafety professional evolved, the biosafety community initiated an effort to define and articulate the knowledge and skill sets essential to biosafety practitioners. The goal of this effort was to establish an internationally recognized credentialing mechanism for the biosafety professional. To this end, the American Biological Safety Association (ABSA), the lead biosafety association in the U.S., together with the American Society for Microbiologists National Registry of

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[2] NIH Guidelines for Research Involving Recombinant DNA Molecules

[3] 29 CFR 1910.1030

[4] Select Agent Regulations (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73)

[5] U.S. Department of Health and Human Services (2010) Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th E. HHS Publication No. (CDC) 21-1112.

[6] National Research Council (2011) Guide for the Care and Use of Laboratory Animals, 8th Edition. Washington, DC: The National Academies Press.

Certified Microbiologists (ASM NRCM) has defined criteria and processes for credentialing biosafety professionals either as Registered Biosafety Professionals or as Certified Biological Safety Professionals. According to ABSA and the NRCM, a biosafety professional must have knowledge in key areas and promote and facilitate safe microbiological practices and procedures.

While one of the driving forces for developing the biosafety guidelines articulated in the *Monograph* was the scientific and public concern about the possible risks of then new recombinant DNA technology, and while the *Monograph* itself references the concept and need for "laboratory security," the pressures that arose during the 1990s and increased in the post-9/11 United States to expand institutional and biosafety professionals' roles to include responsibilities in the realms of biosecurity and biosurety are more the result of sociopolitical forces than strictly scientific ones. In this context, the codification of a compliance role, the "Responsible Official," emerged. While most often assigned to a biosafety professional and while encompassing some overlap with the more traditional role of assisting and facilitating biosafety, the Responsible Official's principle role is to ensure compliance with the requirements of the Select Agent regulations as they pertain to the specific institution's select agent inventory and associated research activities. These regulations, which also direct the institution to assign the responsibility to and grant the Responsible Official authority to fulfill this biosecurity and biosurety role, include clear, prescriptive requirements that can be readily referenced.[7] Repetition of these requirements as they relate to select agents and toxins, therefore, would seem unnecessary in any future edition/revision of the *Monograph*. However, technical advancements, not only in the realm of life sciences but also in the physical sciences (e.g., synthetic biology), have blurred the lines that currently separate, from a regulatory perspective, select agents from other infectious agents and toxins. This important development makes more important than ever the establishment of the culture of responsibility, which served as an underlining principle in the *Monograph.*

Key Aspects of the Roles and Responsibilities

[7] 42 CFR §73.9

No matter how essential or how much broader the biosafety professional's roles and responsibilities are currently relative to those described in the *Monograph*, these responsibilities are integrated with and dependent upon other entities and individuals fulfilling their own roles and responsibilities. It is, therefore, insufficient to delineate the biosafety professional's roles and responsibilities in the absence of a larger discussion/description of the institution's overall safety program and the roles and responsibilities of the other members of a research safety team that includes institutional leadership, the scientists, research support staff, other laboratory safety professionals, and appropriate third-party (community, regulatory) representatives.

To be most effective, individuals charged with meeting these responsibilities must be granted the appropriate authority by the most senior level of institutional leadership. But authority can be derived from and/or enhanced by other drivers, whether top-down, such as statutory requirements, or holistically through the establishment of an institutional culture of safety, research integrity and responsibility. Actions for which clear designation of authority is considered essential include establishment and implementation of procedures for the pre-initiation review of proposed research involving the use of biological materials; the audit of on-going research activities as well as the facilities in which these activities occur; the control and management of inventories; incident investigation and implementation of corrective actions, including the suspension or halting of activities in order to mitigate health, safety or security concerns; and service as the institutional representative in interactions with appropriate community and regulatory groups. Actions that should be inherent to the role of the institution, the biosafety officer, and the researchers include risk assessment, development and implementation of safe working procedures (risk mitigation), risk communication and training.

Reflective of the view of the biosafety profession and drawing upon a performance-based approach, the essential roles and responsibilities can be summarized under the six areas described below. For purposes of specific institutional programs or specific categories of research, more prescriptive approaches to the articulation of recommended procedures and actions may be warranted and appropriate.

- Risk assessment and communication: Assessing appropriate work practices and assigning appropriate containment levels at which research should be conducted requires, as noted in the *Monograph*," considerable scientific expertise, as well as scientific judgment." This process must engage all appropriate areas of expertise on an as needed basis. The biosafety professional is uniquely qualified and indispensable to this process, as this individual ideally brings to the discussion not only a background in relevant scientific and technical knowledge, but also an awareness of and ability to effect changes in the institutional infrastructure (both facilities and policies), if needed. This helps to ensure that, before any work is initiated, all concerns about whether the research can be conducted safely and securely have been considered and explained via a dialogue that includes the investigators themselves as well as the many groups and individuals who serve science in a supporting role.

- Education and training: The recommendation in the *Monograph* that the biosafety professional "provide special laboratory safety training" provides a strong foundation upon which is built the current and growing consensus that "safety culture" be integrated into all levels of science education.

- Technical and scientific advice and consultation: Whether, as included in the *Monograph*, this technical and scientific advice addresses "research safety procedures" or "laboratory security," or is reflective of expertise in "basic microbiology and knowledge of biological safety techniques and practices, containment equipment, and engineering principles pertaining to the design and operation of facility safeguards," the institution should ensure that the biosafety professional has an appropriate combination of education, skills and experience to support the full range of research supported by that institution. Further, the institution should strongly encourage, if not mandate, that staff, faculty, administration, and facility managers seek and consider the advice of the biosafety professional in the realm of biological research activities, the development of research

programs and the construction and maintenance of facilities to support that research.

- Emergency response: The *Monograph* recommends that biosafety professionals be assigned responsibility for the development of emergency plans for and the supervision of decontamination activities (accidental spills, personnel contamination) and laboratory accidents, exposures or releases. Today this important role is expanded in that the biosafety professional must be a member of the institution's emergency preparedness and response program, involved not only in actions to mitigate incidents that are limited to the institution's facilities and/or personnel, but also in efforts to coordinate with and provide response support and expertise to community and regional emergency response plans and efforts whenever possible.

- Biosafety program assessment and validation: The *Monograph* references the need for the biosafety professional to ensure that "laboratory standards are rigorously followed," and that the "integrity of containment equipment and facility safeguards" is maintained, indicating that such be achieved through "periodic inspections" and "testing programs." This role persists today but should be viewed as components of a comprehensive, on-going assessment and validation of the biosafety program as well as its integration into the overall institutional safety and security programs of the institution.

- Institutional representation/liaising with the community and regulatory agencies: Serving as an institutional liaison to regulatory agencies was already recognized in the *Monograph* as a role appropriately assigned to the biosafety professional. Today the biosafety professional should be prepared to represent the institution not only in its dealings with the increasing number of regulatory agencies with jurisdiction over institutional research programs but also with the growing number of stakeholders with the shared goal of expanding knowledge and public benefit while protecting

surrounding communities and the environment through the conduct of safe science.

The role of the biosafety professional is critical and broad in scope, but it cannot be viewed as singular or independent. An effective biosafety program depends upon the institution and all of its members, not just the biosafety professional, meeting their responsibilities.

The biosafety profession must continue to evolve its role to facilitate and support research. It must strive to achieve integration into the academic programs established to educate current and upcoming generations of scientists by serving as credible consultants, and being recognized as knowledgeable advisers to those who conduct research as well as to those who build, operate and maintain research facilities. It is recommended that biosafety professionals expand their already essential role of risk assessment, making available this critical expertise throughout all stages of research, from the development of research proposals, to the design and execution of experimental approaches and continuing through the reporting of scientific results and findings which serve as the basis of scientific knowledge. It is further recommended that the biosafety profession strengthen its commitment to the conduct of safe science via promotion of a professional ethic to establish a culture of responsibility to mitigate potentially adverse events associated with the misuse, whether accidental or intentional, of biohazardous materials in research. And lastly, it is hoped that, in accepting biosecurity and/or biosurety compliance roles, whether statutory or otherwise, the biosafety professional will apply the tenets that have proven so successful to the development of effective biosafety programs, namely risk assessment and communication, collegiality and collaboration, and a strong commitment to support of safe science.

2

Supporting Safe Science

Emergency Procedures

For emergency procedures, the philosophy is plan, practice, perform, and improve. Accidents occur in the course of working with hazardous materials, whether they are biological materials or other hazardous agents within the laboratory. Incidents also result from the failure of equipment or facility safeguards (e.g., leaks, floods, failure of back-up systems for power, water and gas, and building systems failures such as steam and cooling systems). If a failure that impacts containment does occur, use common sense – stop work with potentially hazardous materials and safely contain these materials if possible. In the case of serious injury or illness, the supervisor or principal investigator should determine whether to override containment exit procedures. The likelihood of severe injury or infection can be reduced if plans for emergencies are established and communicated. It is important to remember that it is not possible to recommend a single plan of action. The basic principles for accidental spills in a laboratory environment are well established. Everyone should immediately leave the affected area and not reenter the area until the extent of the hazard is determined, ascertain the necessity for treating exposed persons and then decontaminate the affected area.

Having current emergency plans in place for fire, explosion, and natural disaster is important and must be appropriate for the type of work being conducted. Response plans for natural disasters, severe weather, security, and terrorism must also be considered. These response plans should be integrated into the risk management program of a facility because it provides for a better understanding of the unique risks, supports resource allocation based on priorities and enhances the potential for collaboration. The plan should ensure that operations-specific hazard vulnerability assessments (HVA) on the facility are

conducted. The objectives of the HVA are to help prioritize the risks and impacts, account for unique aspects of facility such as geography, climate, location, construction and dependencies and resources such as redundancy (back-up) and community resources. The HVA should be adjusted based on experience.

Assuring that the facility is properly constructed and commissioned enhances prevention and mitigation of emergencies. Both equipment and systems must be certified and maintenance and operation procedures and contingency plans must be developed. Laboratories can manage the risks; not by just "don't do it" but with the philosophy of "do it better or more safely." Reducing the working volumes and increasing the controls can accomplish this. Developing staff competencies through training, mentoring and "certification," understanding the expectations, and conducting exercises or drills for incident response further promote the prevention and mitigation of emergencies. Developing staff competencies by sharing information and discussion of topics helps promote "ownership" of the processes. The internal response capability must be assessed for addressing spills (by maximizing and optimizing internal capabilities), utility failures, building systems, fires and security.

When planning the external response, consider two questions: What type of support is needed for external response needs? What is the need or scope of support? External support is not likely needed for a spill if a good mitigation plan is in effect. For utility failures, support may be needed for systems servicing the building but not inside the building. For security, it is paramount to train external responders. For building systems, vendor access may be needed. For fire support, there must be a dialogue with response personnel in order to earn their trust as described below. Facility

"Prevention and mitigation are key elements in any emergency management and preparedness program, and must be addressed first. When I look at prevention and mitigation, I start with the people. Developing staff competencies is one of the most effective steps we can possibly take."

– Wayne R. Thomann, Dr. PH

employees are essential during a fire response. They can reduce the risk through a work practice response (by securing agents before evacuation and maintaining personal safety to minimize need for "rescue"), meeting and supporting responders and preparing the "exit" strategy.

Have emergency procedures changed since the 1978 *Laboratory Safety Monograph* publication? Most definitely. Specifically, the emphasis has shifted to proactive management rather than a reactive response. Prevention and mitigation are key elements of emergency management planning. We must also consider recovery from the incident and business continuity so that science may continue. A Business Continuity Plan provides for minimizing the loss of the science (backing-up research material and data) and identifying alternative research options for facilities, equipment and collaborations. A risk-based approach is critical.

The emergency procedures processes can be prescribed but they must not be prescriptive - the approach must be performance-based. Drills and exercises should be tailored to the risks of the agents and procedures. They must be understood and followed routinely. However, they must allow for modification based on actual conditions. The "one size fits all" approach is counter-productive in a laboratory environment because of the variations in risk.

A discussion of the incident command system should be included and adopted both for internal incidents and also training for potential external incidents, i.e., when outside HAZMAT and first aid police are summoned for assistance. At present, there is a move in the U.S. to follow the National Incident Management System (NIMS) for any response if the facility is receiving federal funds. The NIMS provides an integrated framework that defines the roles and responsibilities of federal, state and local first responders during emergency events. It also incorporates the best practices and lessons learned from recent incidents. Many research facilities are affiliated with hospitals or health systems and have a consistent standard of practice. Consideration should be given to mutual aid agreements that support surrounding communities and counties. A model should be used that is based upon risk, such as NIMS.

Risk communication is an important aspect - specifically communication between the facility and the surrounding community.

Who should be the communicator or "voice" of the facility - perhaps the Public Affairs Officer or Director of the facility? Regardless, the individual must deliver a consistent message, support the impacted staff, address facility concerns, address community concerns, and communicate with regulators and public health officials.

Periodically, tours, drills or exercises should be conducted with local fire and police jurisdictions. An annual review and tour of the facility attended by local politicians and local fire, police, and emergency response personnel are warranted to familiarize these individuals with facility operations. Round-table type discussions should include facility administrative (e.g., Director, Public Affairs), security, safety, medical, and engineering personnel. In preparation for a drill, it is important to provide response personnel the details of the microbial agent(s) being used in the facility, what the relative risks are, what protective equipment they have, and how they will be supported if they have concerns about a potential exposure. Response personnel should be provided with a Biological Agent Summary Sheet ('Safety Data Sheet') for each microbial agent being used in the facility. It is important to build confidence with response personnel and earn their trust and respect. The institution's commitment to their safety must be reinforced - especially during an actual incident.

Both for an exercise or actual incident in a Biosafety Level 3 or Level 4 containment environment, emergency response personnel would respond to a medical extraction request wearing Level A full protective gear with self-contained breathing apparatus and other appropriate protective clothing. Level A, being totally encapsulated, is used because response personnel would enter an environment of unknown atmosphere. In most situations, laboratory workers will be stabilizing, containing, and preventing any release of the agent(s). After decontamination of response personnel and the injured person, the injured person would be transferred to local emergency medical services personnel for additional life support measures and subsequent transport to a medical treatment facility.

The incident commander (IC) is the person responsible for all aspects of an emergency response. The individual is responsible for quickly developing incident objectives, managing all incident operations, application of resources as well as responsibility for all persons involved.

The IC in emergency response situations must be quickly identified. The individual must be knowledgeable of the facility and situation - this is where communication becomes a challenge. One of the first things the IC will do is look for someone they recognize and know. There is comfort and trust if they are familiar with the biosafety professional. Therefore, having the biosafety professional participate with the community is very important in cultivating that relationship. Reaching out to the fire community and providing opportunities for response personnel to become familiar with the facilities helps to ensure that they will respond immediately. Some municipalities do it for every building in their "first do" area. Having an incident commander who is familiar with the facility can greatly reduce risks.

It is imperative that an after-action review be conducted following each incident or training exercise - one quickly learns that each exercise is different and provides a new learning experience. Most importantly, these exercises serve to reduce the apprehension harbored by many emergency response personnel because of their unfamiliarity with the medical research or diagnostic environment. There must be a mechanism whereby emergency response personnel can reach back, at any time, to the facility for additional information.

Lastly, an after-action review must be documented. The review should capture accurately and correctly what occurred and include a summary of how the incident was resolved, or an evaluation of the training exercise. The information should be shared with the director and other administrative officials, including the Safety Office and Safety Committee, and the external emergency response personnel.

Risk Communication

A risk communication plan should be established between the institution and the local hospital emergency room (ER). The plan should define the roles and responsibilities of the laboratory worker, the principal investigator, the institution, the receiving facility, the emergency responders and any others who may be involved in the event of injury, illness, or exposure of a laboratory worker. A laboratory workers who suspects that he or she has sustained an exposure to a human pathogen in a laboratory either as a result of an injury or because of symptoms

should report their concern to their principal investigator and to the identified healthcare provider for the facility. If a laboratory worker who suspects a work-related exposure reports to the ER, it is imperative that he or she immediately inform ER personnel of the agent with which they were working, and the suspected exposure. The researcher should be totally familiar with the microbial agent that he or she is working with and provide ER personnel with as much information as possible. Having a Medical Emergency Card or Medical Alert Card to show ER personnel is most advantageous. The disinfection or decontamination procedures that were immediately done following a potential exposure must also be communicated to ER personnel. For example, was a medical emergency bite kit used for immediate treatment? Also, it may be necessary for a facility medical provider to accompany or meet the injured researcher at the ER. This individual could talk to the head of the department, offer to provide their staff an in-service, and provide them with facility medical procedures and biological agent literature. In addition, the accompanying facility medical provider may wish to obtain a specimen(s) from the injured researcher for analysis at the 'home' facility.

It can be unfortunate when the flow of information concerning incidents originates from sources other than the Safety Office or official channels (e.g., Public Affairs). All incidents must be investigated and documented for accuracy and correctness as soon as reasonable after the incident, and shared with the director and other administrative officials (e.g., Public Affairs), the Safety Office and the Safety Committee. Individuals that should be involved with the investigation may include a member of the Safety Office, Security Office and medical staff (a physician and an epidemiologist). Depending on the facility, consideration should be given to sharing this information with the treating medical facility. It may provide a valuable lessons-learned opportunity.

2

Supporting Safe Science

Medical Support Services

In retrospect, the *Laboratory Safety Monograph* guidance for medical support is misleading, inaccurate, and incomplete. First, the clinical services described in the *Monograph* do not constitute medical surveillance. Medical literature does not support the contention that there is value to performing rote physical exams and diagnostic tests[8],[9],[10], storing serum other than at the time of a potential exposure[11], or investigating major or extended illnesses among lab workers. Secondly, it is difficult to defend the assertions that either the "project size" or the availability of medical facilities should mitigate the institution's responsibility to insure that appropriate medical support is available. Finally, the *Monograph* does not address the institution's need to anticipate exposures and provide medical support for work related injuries and illnesses when potential exposures to human pathogens occur.

Medical surveillance, as defined by Alexander Langmuir, M.D., the founder of the CDC's Epidemic Intelligence Service, is the continued watchfulness over the distribution and trends of incidence through the systematic collection, consolidation, and evaluation of morbidity and mortality reports and other relevant data together with timely and

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[8] Fletcher SW, Spitzer WO. Approach of the Canadian Task Force to the periodic health examination. *Ann Intern Med.* 1980; 92:253-4.

[9] Periodic health examination: a guide for designing individualized preventive health care in the asymptomatic patients. Medical Practice Committee, American College of Physicians. *Ann. Intern. Med.* 1981; 95 (6): 729–32.

[10] US Preventive Services Task Force. Guide to Clinical Preventive Services: Report of the Preventive Services Task Force 2nd ed. Baltimore, MD: Williams & Wilkins; 1996.

[11] *LIAR*

regular dissemination to those who "need to know"[12]. Medical surveillance requires a test that is acceptable, reliable, sensitive (few "false negatives"), specific (few "false negatives"), and detects injuries sufficiently early to make a difference. The effort should result in the systemic collection of data and comparison to community norms. Medical surveillance as described in the *Monograph* fails to meet any of the elements of the definition for the activity. Rather than continuing to refer to medical support for biological research laboratories as medical surveillance, this activity should be retitled medical support services.

Medical support services for researchers must be tailored to the health hazards in the institution. If the institution intends to conduct research that would require the use of Biosafety Level 3 (BSL-3) or Biosafety Level 4 (BSL-4) or ABSL-3 (Animal BSL-3) or ABSL-4 (Animal BSL-4) laboratories, then it is especially important that the medical support services be designed and implemented in advance of the initiation of the research. The goal of the services is to enhance the health and safety of the researchers and the community, by preventing laboratory acquired infections (LAIs) and the transmission of these agents to others. To be effective, the provider of these services will need to anticipate and plan for potential exposures to the biologic agent involved in the research. The planning will involve: identifying knowledgeable infectious disease specialists and subject matter experts; developing a mechanism to safely transport the worker for evaluation; a prior agreement with a laboratory and medical facility that can assist with an investigation; and familiarity with community public health resources and plans to communicate with them. Once designed, the services must be available to all workers in the laboratory. Workers who are not employed by the institution must have access to

"Ideally, the services offered in a medical support program are tailored to address the institution's health hazards. And, as is the case in safety, a risk assessment is the cornerstone of any proper medical support program."

– James M. Schmitt, M.D., MS

[12] Langmuir, AD. The surveillance of communicable diseases of national importance. NEJM. 1963, 268; 182-92.

services that are equivalent to those provided to the institution's employees. Barriers to receiving the services must be minimized, so that workers can access the service promptly, 24 hours a day.

Medical support services typically consist of the following basic elements: preplacement medical evaluations; routine, periodic medical evaluations; medical care for work-related injuries and potential LAIs; emergency response for medical issues that may occur in the laboratory; and participation in ongoing training and drills related to the work preformed or planned for the laboratory. The following is a brief description of these services.

The preplacement medical evaluation, by definition, occurs before the worker initiates work in the laboratory or is permitted to have unrestricted access to the biologic hazards stored in the laboratory. The visit permits an introduction of the laboratorian to the medical staff that will provide medical care in the event of a laboratory accident. The clinicians take the opportunity to learn more about the worker's role and responsibilities in the laboratory. The healthcare provider collects information to determine whether the worker can assume the duties of the position without unacceptably jeopardizing his own health and safety or someone else's. The clinician is alert for conditions that could result in: an altered level of consciousness; impaired judgment or concentration; inability to utilize personal protective equipment; inability to perform the physical requirements of the position or reliably comply with safety guidance; or an increased risk of serious injury, if the worker were exposed to the biologic hazard. The provider also inquires about the worker's personal medical history (e.g., current illness, treatments, allergies, and a review of organ systems; prior illnesses, surgeries, traumas, and immunizations) and social history (e.g., personal use of alcohol and drugs, who lives at home and their health, and the worker's hobbies). The provider may perform limited testing to form an opinion of whether the worker is physically and emotionally capable of performing the duties of the position and will adhere with guidance on safe work practices. Based upon the information that is obtained, the healthcare provider offers work-related immunizations, other immunizations to prevent common febrile illnesses (e.g., influenza), and counseling. The counseling consists of: advising the worker to report all work-related injuries and each febrile illnesses; a detailed description of

first aid measures and necessary steps to access medical care immediately for suspected occupational exposures to biohazards; a description of the earliest presenting symptoms of a LAI involving the agent studied in the laboratory, and the provision of agent-specific informational handouts and a wallet card.

Workers are recalled annually. During these routine, periodic evaluations, the healthcare provider: obtains interval occupational, medical and social histories; reviews and updates work-related immunizations; and reiterates the counseling provided at the initial visit.

Laboratory workers must immediately report all work-related injuries and unexplained fevers. The healthcare provider obtains a detailed description of the circumstances of the incident from the worker, and, if relevant, from the lead investigator and the safety specialist assigned to the laboratory and examines the worker. Ideally, the healthcare providers providing support for these incidents will have agreed upon a well-defined system for rating the risk of transmission in advance of an incident. If an exposure to a human pathogen is considered a possibility, the clinician consults with knowledgeable infectious disease specialists and subject matter experts. Collectively, they determine how the worker's activities should be limited and whether diagnostic testing is warranted. If there is any concern that the worker could have sustained an exposure to a human pathogen, blood is drawn from the worker and the sera is stored in a non-frost-free freezer as an acute specimen. If further evaluation is clinically warranted at the time of the incident, the mechanism for transporting the worker to the previously identified facility for testing and care is initiated. At this point, medical responsibility for the worker typically is transferred to infectious disease specialists. Each of the participants in this process, starting with the worker, should have an understanding of his or her role, so that an expert evaluation is initiated as efficiently as possible and the potential risk to others is minimized to the fullest extent possible. Appropriate institutional officials must be informed of the events, as they unfold, and they in turn must decide whether and when community public health officials are notified of the occurrence. Throughout these events, it is useful to have someone assigned to maintain a log of events for future reference.

The facility receiving the worker will require an effective strategy for moving the patient through the building and providing the needed care, including processing specimens, safely. At an interval appropriate for the biologic hazard of concern, a second specimen is obtained and stored. If clinically warranted, the smallest possible volume necessary for testing is submitted for testing. The specimens are coded to blind the laboratory as to the date the specimen was obtained. Positive and negative serum specimens may be submitted and all specimens are tested simultaneously.

Medical emergencies in laboratories, unrelated to work-related accidents, are an infrequent occurrence. However given the potentially grave consequences of such an event, every effort is made to minimize the likelihood of these incidents and plan for handling them should they occur. Both during the preplacement and annual medical evaluations, the medical provider attempts to identify workers who may not be medical fit to work in a laboratory environment. If despite these efforts, a sudden medical event occurs in the laboratory, the worker is rapidly decontaminated, removed from the laboratory and emergency medical efforts are initiated outside of the laboratory.

Training for response personnel is an ongoing process and needs to include information about the biohazards used in the research, the earliest presenting signs and symptoms of a LAI with the agent, and the incident response plan. If possible, the training should involve as many of the parties likely to be involved in an incident, such as the researchers and their support staff, the safety specialists, the occupational medical and infectious disease specialists, and public health officials. Training should reinforce the material provided in the training and test various aspects of the response plan. Examples of issues addressed by drills include: first aid following an incident; activating the emergency response system: the effectiveness of communications among the various partners in the response plan; handling illnesses that are recognized during off duty hours; transporting the ill or injured worker; moving the worker through the treatment facility; plans for performing diagnostic tests; a review of the therapeutic options including access to treatments that are not commercially available; and the plan for an injured worker who declines to follow the medical advice provided. As quickly as possible following the conclusion of each drill, the participants should

meet and review what did and did not work, so that the response plan can be improved.

The cost associated with developing and maintaining competent medical support services for research in BSL-3 and BSL-4 and ABSL-3 and ABSL-4 facilities is significantly higher than the average cost of medical support services for almost any other type of work. However, when those costs are compared to the financial and other costs associated with a mishandled LAI, the expense should be acceptable.

2

Supporting Safe Science

Biological Safety Training

Ascendancy of Biosafety Training

There has been enormous growth in the discipline of biosafety training since publication of the 1978 *Laboratory Safety Monograph*. This is due to several key factors such as new federal regulations mandating specific training for conducting research involving high-risk pathogens, scientific advancements, a new emphasis on biosecurity, and an elevated interest of creating and sustaining a culture of safe and responsible research. Hundreds of biosafety training opportunities now exist and the breadth and quality of the training continues to expand and improve.

"From the very beginning of biosafety training at Fort Detrick in the 1940's and through today, the strongest programs evolve when senior management sets teaching safety and personal accountability as a top priority, principal investigators nurture safe behavior, and laboratory workers commit to conducting research in the safest manner possible."

– W. Emmett Barkley, Ph.D.

Contrast this with the training section of the 1978 *Monograph*, which included a list of most all training aids and courses available at the time. The section identified nine safety training "slide-tape cassettes" offered for a fee from the National Safety Council or the National Archives Trust Fund. The tapes covered such topics as *Assessment of Risk in the Cancer Virus Laboratory* and *Basic Principles of Contamination Control*. In addition, 12 "films" were referenced, available for loan from the National Audiovisual Center. They addressed *Controlling Infections Aerosols, Parts*

1 and 2 and *Plastic Isolators: New Tools for Medical Research*. Three training manuals for animal caretakers were cited, available from the American Association for Laboratory Animal Science, and Ralston-Purina Company.

Under the heading of "Training Courses," four were listed:

- *Laboratory Safety Management,* presented by CDC, Atlanta
- *Safety in the Laboratory,* presented by the National Institute of Occupation Safety, Cincinnati
- *Biohazard and Injury Control in the Biomedical Laboratory,* presented by the NCI, Office of Research Safety (ORS), NIH
- *Biohazard Containment and Control for Recombinant DNA Molecules*, presented by the NCI, ORS, NIH

Fundamentals for Safe Microbiological Research

Concurrent with the publication of the 1978 *Monograph*, the NIH Division of Safety and the National Institute of Allergy and Infectious Diseases (NIAID) joined together to support the University of Minnesota and the American Society for Microbiology to develop training materials intended to provide a minimum base of knowledge and skills that any individual working with potentially biohazardous agents or with recombinant DNA molecules should demonstrate. This project was undertaken in response to questions and concerns about biological safety that arose from ongoing recombinant DNA controversies. This collaboration produced *Fundamentals for Safe Microbiological Research,* published in 1979.

Fundamentals for Safe Microbiological Research included five units. Unit I, Host-Parasite Relationships addressed the nature of micro-organisms; factors in pathogenicity and virulence; factors in invasiveness and infectivity; the "Infection Chain;" reservoirs, portals of escape and entry into new hosts; natural host defense mechanisms; host immune status; antibiotics and antibiotic resistance; and History and Prevalence of Lab-acquired Infections.

Unit II, Microbial Ecology, addressed Natural Environments (Water, Soil and Air); Transport and Dispersal; and Establishment or Colonization by Introduced Species.

Unit III, Principles of Physical and Chemical Containment, addressed Basic Principles of Contamination Control; Dissemination of Microbes from Standard Laboratory Procedures; Primary Barriers; Secondary Barriers, Packaging and Shipping of Etiological Agents; Equipment Design for Safety; Decontamination and Sterilization – Heat Treatments; Decontamination and Sterilization – Liquid Chemical Germicides; Decontamination and Sterilization – Gaseous and Ultraviolet Treatments; Waste Disposal; Emergency Plans; Storage, Packaging and Shipping of Etiological Agents; and Personal Protection and Personal Hygiene.

Unit IV, Biological Containment for Recombinant DNA Molecules, addressed the NIH Guidelines for Biological Containment in Recombinant DNA Research; E.coli Host/Vector Systems; Non-E. coli Host/Vector Systems; and Eucaryotic Cell Vectors.

Unit V, Laboratory Skills – Including Hands-On Laboratory Exercises, addressed Basic Techniques in Microbiology including 1) aseptic technique, 2) isolation of pure cultures, 3) staining, 4) dilutions, pour plates, and counting procedures, 5) working in biological safety cabinets, 6) decontamination and sterilization, and 7) laboratory animal handling practices.

Fundamentals for Safe Microbiological Research became a cornerstone for biosafety training, and the fundamentals for safe practices described in the text reflect the framework for maximum containment laboratory training that continues to develop today.

Collaborating Center for Applied Biosafety Programmes and Research

In 1983, the World Health Organization (WHO) selected the NIH Division of Safety to be a Collaborating Centre for Applied Biosafety Programmes and Research. The WHO recognized the need to build an international cadre of well-trained biosafety instructors with the expertise and materials to deliver uniform biosafety training worldwide. The Division of Safety was tasked with developing a "train-the-trainer manual," which would serve as an instructor's guide for biosafety training. *Laboratory Biosafety Principles & Practices – An Instructor's Guide for Biosafety Training* was published in 1983. The *Guide* drew heavily from the *Fundamentals for Safe Microbiological Research*. In 2007, the NIH

Division of Occupational Health and Safety (DOHS), formerly the Division of Safety, produced a revised *Instructor's Guide for Biosafety Training* that updated and expanded much of the information found in the 1983 *Guide*.

42 CFR Part 73 – Select Agents and Toxins

In 1995, Congress directed the Secretary of Health and Human Services (HHS) to establish a list of biological agents and toxins that have the potential to pose a severe threat to public health and safety. It required, through regulation, that HHS establish procedures for the transfer of these agents and set uniform safety standards for entities performing these transfers, including among other things, ensuring that entities have the appropriate training and skills to handle those agents safely, and that the laboratory facilities have the proper containment and destruction protocols available for those agents.[13] HHS delegated the Centers for Disease Control and Prevention the responsibility for implementing the regulation. That regulation became known officially as 42 CFR Part 72.6 titled "Additional Requirements for Facilities Transferring or Receiving Select Agents." Part 72.6 was superseded on March 12, 2003, by the Interim Final Rule, 42 CFR Part 73, published in December 2002, and known as the new Select Agent Regulation.

Part 73.12 Biosafety, of the new Select Agent Regulation included language mandating a written biosafety plan as follows:

> "An individual or entity required to register under this part must develop and implement a written biosafety plan that is commensurate with the risk of the agent or toxin, given its intended use. The biosafety plan must contain sufficient information and documentation to describe the biosafety and containment procedures.
>
> The biosafety and containment procedures must be sufficient to contain the select agent or toxin (e.g., physical structure and features of the entity, and operational and procedural safeguards).

[13] Hempill, M.L. *Oveview of the New CDC Select Agent Rule: Title 42 CFR Part 73*. Applied Biosafety, 9(2) pp.88-96©ABSA 2004.

In developing a biosafety plan, an individual or entity should consider the CDC/NIH Publication *Biosafety in Microbiological and Biomedical Laboratories*; the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines), and the Occupational Safety and Health Administration (OSHA) regulations in 29 CFR Part 1910.1200 Hazard communication and 29 CFR Part 1910.1450 Occupational exposure to hazardous chemicals in laboratories.

The plan must be reviewed annually and revised as necessary. Drills or exercises must be conducted at least annually to test and evaluate the effectiveness of the plan. The plan must be reviewed and revised, as necessary, after any drill or exercise and after any incident."

In addition, 42 CFR Part 73.15 Training included specific biosafety and security training requirements, as follows:

> "An individual or entity required to register under this part must provide information and training on biosafety and security to each individual with access approval from the HHS Secretary or Administrator before he/she has such access.* In addition, an individual or entity must provide information and training on biosafety and security to each individual not approved for access from the HHS Secretary or Administrator before he/she works in or visits areas where select agents or toxins are handled or stored (e.g., laboratories, growth chambers, animal rooms, greenhouses, storage areas, etc.). The training must address the particular needs of the individual, the work they will do, and the risks posed by the select agents or toxins.
>
> Refresher training must be provided annually. A record of the training provided to each individual must be maintained. The record must include the name of the individual, the date of the training, a description of the training provided, and the means used to verify that the employee understood the training."

* The training need not duplicate training provided under the OSHA Bloodborne Pathogen Standard set forth at 29 CFR 1910.1030.

National Biosafety and Biocontainment Training Program

In 2004, the NIH DOHS created the National Biosafety and Biocontainment Training Program (NBBTP) as a partnership with the NIAID. Its mission is to prepare biosafety and biocontainment professionals of the highest caliber to meet the needs of the biomedical, emerging disease, and civilian biodefense research communities through the 21st century.

Fellows train specifically to support high containment research environments by acquiring knowledge and skills necessary to meet the scientific, regulatory, biocontainment, biosafety, engineering, communications, management, and public relations challenges associated with the conduct of research in these facilities. Core areas of concentration include:

- Public Health
- Applied Biosafety and Biocontainment
- Integrated Occupational Health
- Biological Sciences
- Biosecurity

Alumni of the NBBTP currently hold leadership positions in biosafety and health and safety program management nationwide including Biological Safety/EHS Manager of the Regional Biocontainment Laboratory in Louisville, Kentucky; Associate Director, High-Containment Safety for the National Emerging Infectious Disease Laboratories at Boston University Medical Center; Biosafety Officer for the University of Pittsburgh; Associate Biological Safety Office for Cornell University; Associate Biosafety Officer at the NIH; Associate Biosafety Officer at the Rocky Mountain Laboratories in Hamilton, Montana; Biosurety Training Program Manager at the NIH; Biosafety Specialist at the Biosecurity Research Institute at Kansas State University; Biosafety and Biosecurity Specialist at the National Biodefense Analysis

and Countermeasures Center at Ft. Detrick, Maryland; Biosafety Manager for the NIH NIAID Integrated Research Facility at Ft. Detrick, Maryland; Maximum Containment Laboratory Training Center Manager at the NIH; AAAS Science and Technology Policy Fellow; Regulatory/Environmental Health and Safety Specialist at the J. Craig Venter Institute; and Fellowship Program Director for the NBBTP in Bethesda, Maryland.

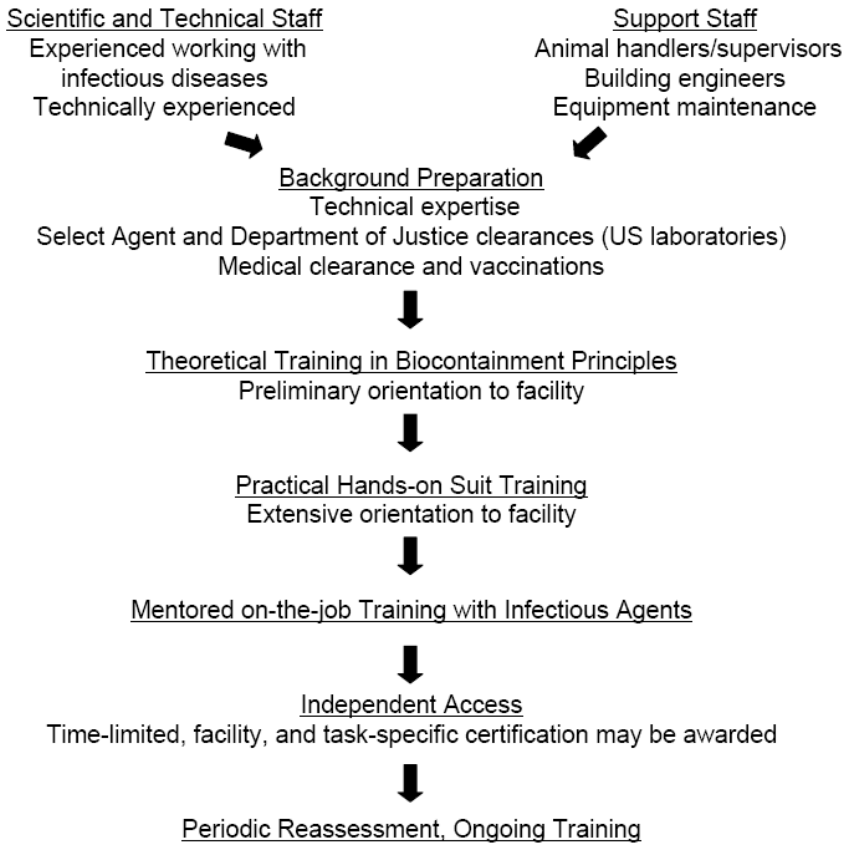
Framework for Leadership and Training – BSL-4

In 2008, a group of renowned biomedical researchers recognized that the construction of several new Biosafety Level 4 laboratories and expansion of existing operations had created an increased international demand for well-trained staff and facility leaders. Directors of most North American BSL-4 laboratories met and agreed upon a framework for leadership and training of biocontainment research and operations staff. They agreed on essential preparation and training that includes theoretical consideration of biocontainment principles, practical hands-on training, and mentored on-the-job experiences relevant to positional responsibilities as essential preparation before a person's independent access to a BLS-4 facility. They also agreed that the BSL-4 laboratory director is the key person most responsible for ensuring that staff members are appropriately prepared for BSL-4 operations. Although standardized certification of training does not formally exist, the directors agreed that facility-specific, time-limited documentation to recognize specific skills and experiences of trained persons was needed.

The article, "Framework for Leadership and Training of Biosafety Level 4 Laboratory Workers," was published in the *Emerging Infectious Diseases* Journal in November.[14] The article stressed the importance of increasing the number of scientists with expertise in biosafety to promote safe science within the BSL-4 containment facilities. A "Framework for Maximum Containment Laboratory Training" appeared in the article. Interestingly, it reflects much of the biosafety training guidance provided in the 1978 *Fundamentals for Safe Microbiological Research*. The "Framework for Maximum Containment Laboratory Training" follows.

[14] LeDuc, James W., et al. 2008. *Framework for Leadership and Training of Biosafety Level 4 Laboratory Workers*. Emerging Infectious Diseases Journal. Vol. 14, No. 11.

Framework for Maximum Containment Laboratory Training



Guidelines for Biosafety Training Programs – BSL-3

In 2013, a group of biosafety professionals who oversee training programs for the two national biocontainment laboratories (NBL) and 13 regional biocontainment laboratories (RBL) that participate in the NIAID NBL/RBL Network developed *Guidelines for Biosafety Training Programs for Workers Assigned to BSL-3 Research Laboratories*. These guidelines provide a general training framework for Biosafety Level 3 high-containment laboratories, identify key training concepts, and outline

training methodologies designed to standardize base knowledge, understanding, and technical competence of laboratory personnel working in high-containment laboratories. Emphasis is placed on building a culture of risk assessment-based safety through competency training designed to enhance understanding and recognition of potential biological hazards as well as methods for controlling these hazards. The "Guidelines" were published in *Biosecurity and Bioterrorism*. They provide valuable guidance to institutions and academic research laboratories that develop biosafety training programs for BSL-3 research.[15]

A Training Program in Practice

One example of a successful and robust biosafety training program is in place at the University of Texas Medical Branch (UTMB). The UTMB Laboratory Biosafety Training Program (LBTP) offers training in biosafety for all laboratory workers who are actively working, or have accepted employment in a laboratory for BSL-2, BSL-3, and BSL-4. The program establishes a solid base of laboratory skills and application of biosafety principles. The LBTP courses are designed to provide initial training for workers entering BLS-2 through BSL-4, or for experienced BSL-2 through BSL-4 workers who would like to be refreshed in proper techniques specific for their appropriate biosafety level. The training is comprised of an initial assessment of biosafety theory and practical application of laboratory bench skills. Once completed, the assessment provides the training template. After both phases of training – theory and practicum – are completed, a final theory and practicum assessment is conducted. The trainee receives a certificate of training and begins mentorship with their respective institutions.

The Assessment Phase

A written test is administered to the trainee focusing on the proper use of a biological safety cabinet, personal protective equipment, and related safety topics. A hands-on evaluation follows in which the trainee is

[15] Homer, Lesley C., et al. 2013. *Guidelines for Biosafety Training Programs for Workers Assigned to BSL-3 Research Laboratories*. Biosecurity and Bioterrorism. Vol. 11, No. 1., pp.10-19.

asked to perform related protocols based on their research using appropriate biosafety conditions. The trainer does not intervene during this process and takes notes on both the safety and scientific techniques employed. This is allows the determination of experience and what level of training is required. At the end of the assessment, the trainer reviews the assessment results with the trainee and identifies the specific areas of where the trainee needs improvement.

The Training Phase

This phase is comprised of a theoretical class and practicum. The theory portion of the training emphasizes the biosafety levels, personal protective equipment, proper use of biosafety cabinets, aerosol procedures, emergency procedures, and decontamination and waste management. This training is specific to the biosafety level requested. The practicum compliments and reinforces the theory course, as it allows the trainee to experience difference scenarios in a safe environment. It allows the trainer to observe, advise and correct the trainee's techniques in the lab with respect to safety and in some cases, scientific issues (e.g., contamination of cultures). The practicum is specific to the biosafety level and the agent to be used (e.g., bacteria, parasite, viruses). Pre-course communication with the principal investigator allows the use of specific protocols or facility specific practices, if needed.

The Final Assessment

A final assessment, identical to the initial assessment is conducted and recommendations are made to the principal investigator and trainee. Recommendations include work that the trainee can perform safety and immediately, and areas that need attention.

Return to the Institution and Mentorship

Following a successful completion of the training program, the trainee receives appropriate biosafety level certification. Once agent specific training has been provided, the training begins supervised mentorship with their principal investigator within three months of completing the

course. The mentorship program is an important part of the training process for new laboratory workers. Protocol and site-specific facility training should be included in the supervised mentorship. During mentorship, the trainee must be supervised at all times by an experienced researcher. The duration of the mentorship phase is based on each institution's requirements and takes into consideration the ability of the trainee to adapt to the environment. The mentor, principal investigator and when appropriate, the laboratory director, must be confident in the new laboratory worker's knowledge and proficiency in working with infections materials.

3

Practicing Safe Science

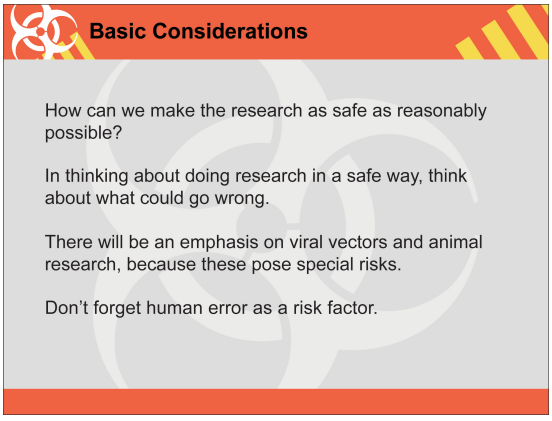
Risk Assessment

"The ultimate message is that it is humans and human error that are the most dangerous part of research. I've never seen a serious lab accident in which the person doing the experiment had a clear, careful, well thought out protocol and followed that protocol religiously."

– Stephen H. Hughes, Ph.D.

Following is the talk, titled "Risk Assessment," presented by Stephen H. Hughes, Ph.D., at the Safety by Design Symposium.

The goal of risk assessment is not to make research risk free. That's not possible. But, we want to make it reasonably safe, and that really becomes an exercise in trying to understand what could go wrong.



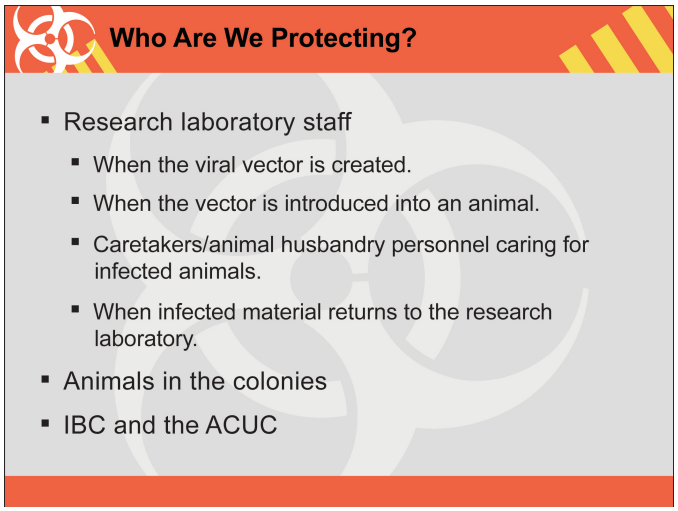
In this discussion, I will emphasize viral vectors in animal research, and that is in part because I have some expertise in that area, but also because in terms of what people often see in IBCs, these are some of the experiments that pose special risks.

One of the things that I am sorry to say we really have to bear in mind is that human error is a substantial risk factor. People make mistakes. They can be careless. And, unfortunately, when someone does something that perhaps is a little thoughtless and gets into trouble, they can react badly to the situation and get into yet additional trouble. That happens all too frequently.

In terms of risk, we also need to think about whom we are actually protecting. Of course, we begin by protecting research laboratory staff. If we are dealing with our vector, we begin when the viral vector is created in the laboratory.

There is also the issue of when the vector is introduced into an animal, if that is part of the experiment. If animals are involved, we not only have the research staff, who ought to be familiar with the risks associated with the vector or the tools that they've created, but animal caretakers, who in many cases, are not familiar with the risks. People somehow seem to forget that if they put something unpleasant into an animal and that animal is then sacrificed, when they bring materials from the animal back into the laboratory, the agent may still be present. I'm regularly astonished by peoples' reaction to that.

To some extent, in thinking about risk, we need to think about the animals' welfare. There ought to be a reasonable interaction between the IBC and the ACUC. In many cases, if there is good interaction, you can learn quite a bit if you are on the IBC, by finding out what the researcher has told the ACUC. That kind of interaction is extraordinarily helpful. Unfortunately, in our experience, what the ACUC is told and what the IBC is told is not always congruent.



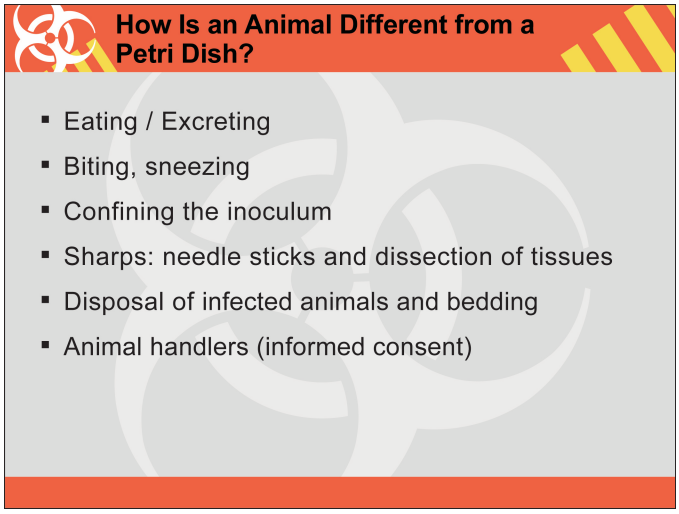
One of the things we also find is that very often researchers who are trained to do viral research do not begin their careers working with animals. They don't think about how an animal is different than a petri dish. It is just where they've begun to do their research.

Animals eat and excrete. You have to worry about what goes into the animal and to a greater extent, what comes out. They bite. They sneeze. Anybody that has worked with animals over the long term has probably been bitten. I've been bitten while handling laboratory mice. It happens. And, if you are talking about introducing a pathogen, particularly a viral vector, you have to worry about how to confine the inoculum before you put it into the animal. Then you have to worry about confining the animal. I'll talk more about that in a few minutes.

When putting something into animals, one of the real serious problems that we run into is sharps. Certainly, there is an obvious problem with needle sticks when people introduce viral vectors or other pathogens into animals with a needle. But there is also the issue, again at the end of the experiment, when the animal is sacrificed and dissected. People use sharps in most cases to dissect the animal, and cutting the animal sometimes leads to cutting the researcher.

And, finally, at the end of the experiment, or sometimes during the experiment, if an infected animal dies or is sacrificed, both the animal and the bedding need to be disposed of in a safe and rational fashion.

I'm going to come back to and emphasize the problem of informed consent with the animal handlers. Animal handlers, for the most part, are not rigorously trained in higher order microbiology. They don't know what they are working with. It is the obligation – and that is the right word – of the researcher, under the supervision of the IBC, to make sure that animal handlers are know and understand what they are dealing with to the extent that they can work in a safe way. Simply saying, "Oh, you don't have to worry," doesn't cut it.



Cells in culture don't sneeze, but aerosols with some types of agents are a serious issue. And although this is meant to illustrate the significance of an animal's ability to bite, it is very often the mice, which are much smaller and less aggressive and probably are handled more often and less carefully, that are most likely to get somebody bitten. So, is it part of the experiment to transfer something from the oral cavity of the animal? If it is, you probably should think about how to avoid that.

In thinking about risk, particularly if we are talking about viral vectors, we need to think about what the vector naturally does and what has been done to change the way the vector behaves. What is the pathogenicity of the parent vector? If the parent vector is HIV, that is serious stuff. Even adeno is not completely benign, and vaccinia, as I'll show you in a few minutes, is certainly not benign.

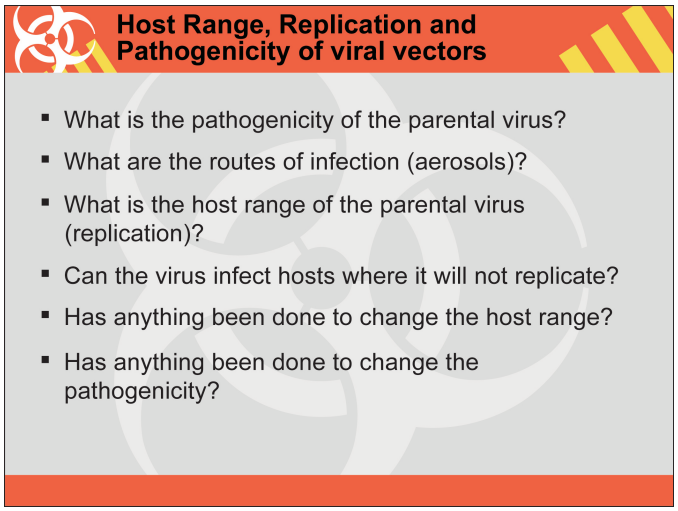
What are the routes of infection? I mentioned this already. Something that is spread, or potentially spread, as an aerosol is much more of a serious potential problem in terms of casual spread than something that is not. In a sense, we have had very few breaks or bits of good fortune associated with the HIV pandemic, but there is one that we should be grateful for: it is not transmitted as an aerosol. If you want to have nightmares, think about what could happen if HIV were ever to develop the ability to be transmitted as an aerosol.

What is the host range of the parental virus? Does it replicate in humans? There are things that will infect humans that are not capable of replicating in humans, and obviously, the design of the average defective viral vector means that it is supposed to infect, but it is not supposed to replicate. However, as we will discuss in a moment, there are exceptions. There are replication competent vectors and there is the potential problem that vectors that are not intended to be replication competent can, in fact, be replication competent. This is the problem I

just mentioned. In some cases, people have tried to use vectors that do not replicate in humans, but can infect humans. That is, in theory, safer.

One of the other things that is an important consideration is, has the manipulation that has led to the creation of the vector changed the host reaction? There are, for example, adenoviruses in which the host range has been manipulated with the intent of extending the host range. In that case, people should be, in fact, more careful.

The thing that really should catch everyone's attention – from the person designing the experiment to the person making sure that the experiment is being done safely – is, has anything been done to increase pathogenicity? I work at the NCI. Very often, people take viral vectors and put oncogenes in them. That for sure is intended to increase the pathogenicity of the vector. Then, that is part of the risk assessment.



Now, the goal of the game, as I said at the beginning, is not to do things that are risk free. We are not in the business of doing things that have no risk. The goal is to be as safe as reasonably possible to make the experiment doable, and of course, to do the experiment in a way that poses the least risk to the participants.

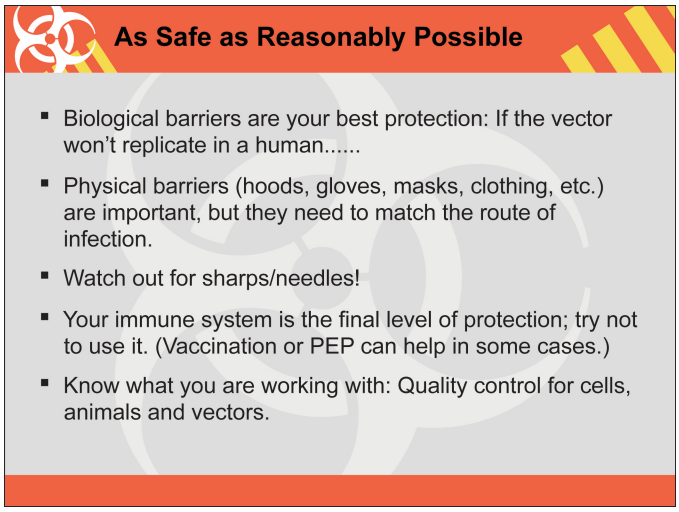
Biological barriers are the best possible protection for the experimentalist. If the person doing the experiment can't get infected, the virus can't replicate in that person. They are going to be pretty safe.

Physical barriers are important, but they need to match the route of the potential infection. Wearing a mask to work with an HIV vector is pointless. Wearing a mask with vaccinia is a really good idea.

I am going to come back to this repeatedly. Watch out if someone says they are going to work with sharps or needles. Sharps and needles are a great way to introduce stuff inside a person where it can hurt them.

Very often we're protected by our immune system. It is the final barrier, but people ought not to try and use it. There are a few cases in which vaccination can help. In fact, it can help against vaccinia. There are a few cases – HIV is one of them – in which post-exposure prophylaxis can help. People should be properly prepared when those levels of protection are available. Unfortunately, in many cases, for many viral-vector systems, we don't have those luxuries.

One of the things that causes continual battles between the PIs and the IBCs is, does the person really know what they are working with? Have they properly characterized the agent that they have – not what they think it is, but what it actually is? One of the wonderful things about modern biology is that we are now in a position where we have much better command in terms of knowing what is in a particular preparation, viral vector, or whatever, than we ever did before. But, of course, that only helps if people actually do the quality controls necessary to define what it is they have.



We are going to go through some special considerations for a few of the viral vectors that people see in IBCs. I am not going to go into this in any great depth because most people don't have call to worry about this in real depth on an average basis.

What about retroviral and lentiviral vectors? One thing that is really important about these vectors, and one of the reasons that people want to use them, is that the viral DNA inserts itself into the host genome. That means two things. First, the infections are persistent because as long as the infected cell survives, the DNA from the virus is there. Second, although people do not worry about this very often, although they have a little bit more since the problems with the gene therapy trials in France, the insertions cause mutations. Putting a piece of viral DNA into a genome is a mutagenic event that can have serious consequences.

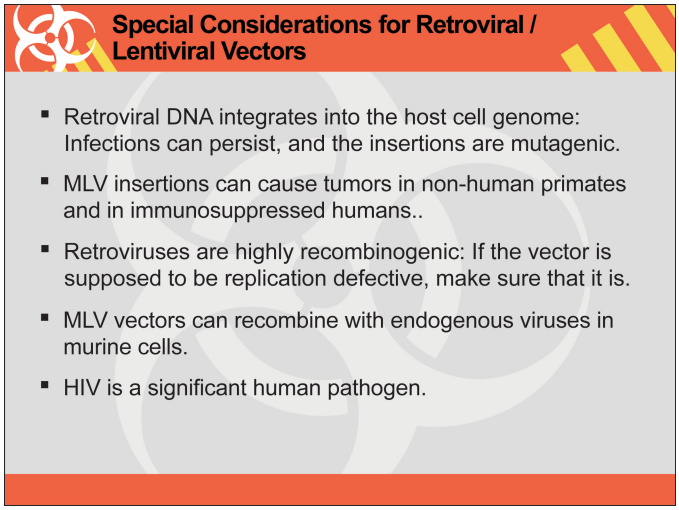
One of the serious consequences that it can have, which was manifest in those French gene-therapy trials, is that the insertions, particularly of an MLV vector, can cause tumors. Those tumors have been discovered in both non-human primates that were used as tests for some gene-therapy experiments, and in immunosuppressed humans where there have been deaths from this kind of oncogene activation.

The second thing that, unfortunately, not every researcher is familiar with is that retroviruses are very recombinogenic. If the vector is supposed to be replication defective, that is to say, if the manufacturer says that they have broken the bits of the viral gene up into pieces and you don't have to worry about replication, it is a really good idea to make sure that it really is replication defective. In many cases, the vectors are well designed and they will be replication defective. But, it is not always true.

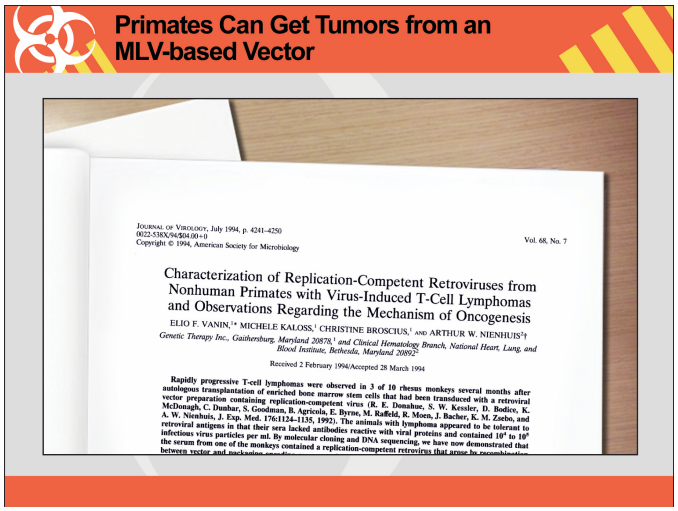
Another thing researchers forget is if you are using a vector that is designed from a murine leukemia virus – a mouse virus – the genome of the mouse contains thousands of copies of related viruses. You can put a virus into a mouse that is replication defective, and the missing pieces of the virus that are necessary to make that virus replication competent, can be supplied by the mouse.

In some ways, a murine vector in a mouse cell is one that people almost never think about. A murine vector in a human cell that is put into a mouse does not have to stay where it is put.

Finally, people are now working as much with lentiviral vectors as they are with murine vectors. HIV is a significant pathogen, and there is some very good and reasonably safe HIV vectors out there. We work with them every day, and you can do those experiments safely. But at the same time, it is appropriate to make sure that the vector behaves as you wish and that it remains replication defective. Even the replication defective versions are quite able to infect humans in human cells.



This is an actual publication, which shows that if you put a replication competent virus into a nonhuman primate, and this is not an immunocompromised nonhuman primate, they can get tumors. Unfortunately, these were done in immunocompromised SCID-trialed patients. You can get tumors as well, and two or three of the children in this particular clinical trial died as a result of oncogene activation by the vector with which they were treated.

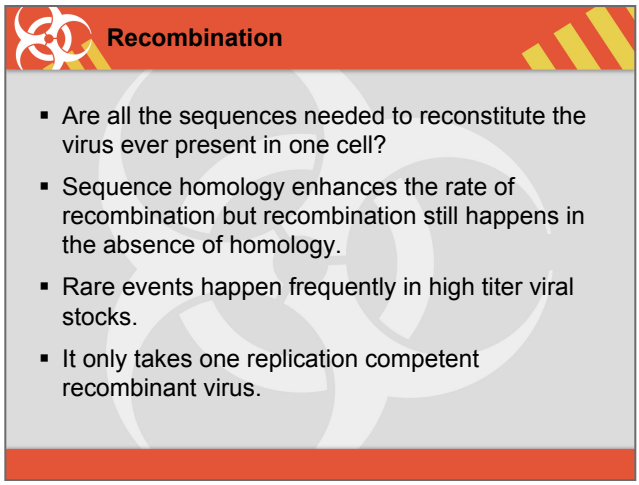


I mentioned a few moments ago that if you put a human cell through a mouse, the human cell can acquire mouse viruses from the mouse. It actually turns out that when people use human cell lines in the laboratory, a surprising number of times they are unaware of the fact that they have replication competent mouse viruses in them. This is a common problem and people overlook it all the time.

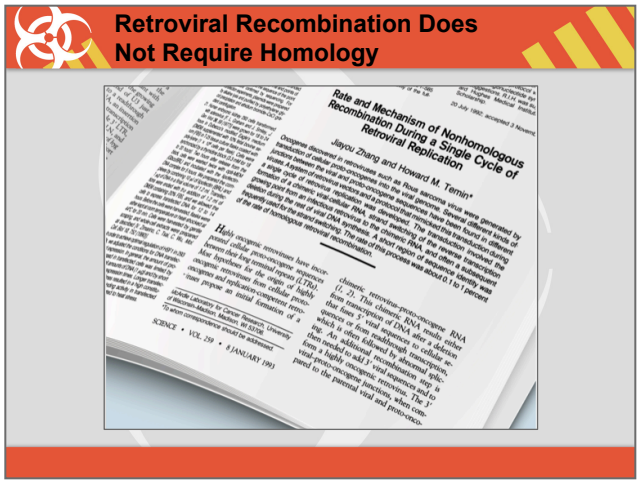
So what about recombination? People talk about the fact that their vector is safe because they have eliminated the regions of homology between the pieces of the vector. That is actually, as I will show you in a moment, not good enough. The real question is in the experiment: Are all the sequences needed to reconstitute the virus present at the same time? If they are, you best watch out.

Sequence homology enhances the rate of recombination, but recombination still happens even if there is no homology. The magic is whether all the pieces are there, not whether there are big overlaps. The reason this is true is because when one works with virtual stocks, one works as hard as possible to get as high a titer stock as possible, and when the titer is high, events that are rare happen frequently. If you have a stock that is ten to the seventh, an event that happens one in a million is virtually certain. An event that is rare does not mean that it is infrequent.

The final thing about grabbing the brass ring in terms of recombination – the important thing to remember – is that it takes one replication competent virus to take over the culture. It only has to happen once. They are replication competent. They grow.



Here is a paper from Zhang Temin showing, in fact, that even if you eliminate homology you get recombination. You can get replication competent viruses out without homology.

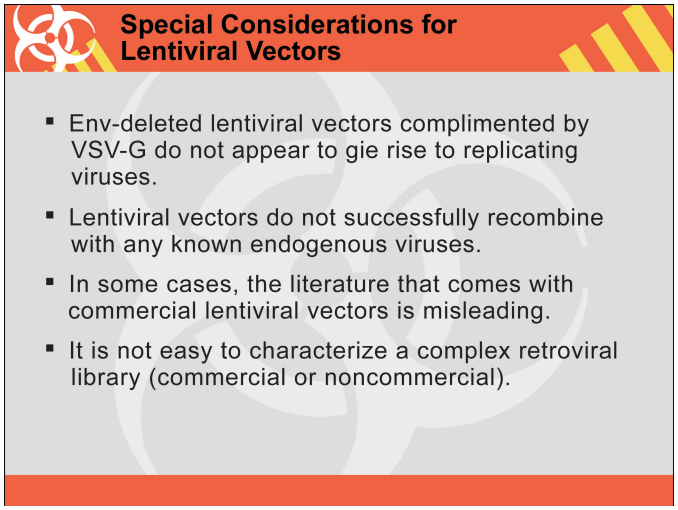


Lentiviral vectors are, in a sense, a specialized subset of retroviral vectors. We are fortunate in that we can use VSVG to compliment the lentiviral vectors, and that combination appears not to give rise to replicating viruses. This is probably because VSVG is hard on the cells.

The other thing that we are fortunate about is the lentiviral vectors do not appear to successfully recombine with endogenous viruses either of humans or of mice to give something that is replication competent. This is in contrast to what happens with a murine vector in a murine cell.

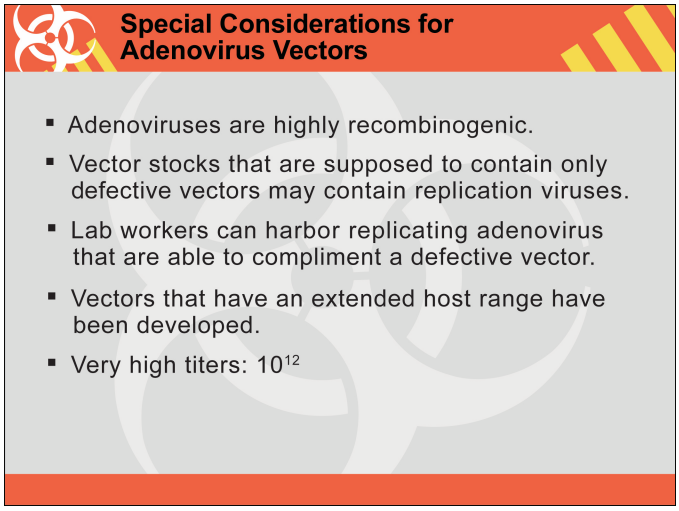
However, I am going to warn everybody that the companies who sell these things put literature in with their products, not all of which is entirely accurate. Sometimes you will have one of your PIs come to you and hold up some of this literature, and it is just not going to be right. I feel bad about this, but it is true.

The final thing to bear in mind is that people are now building very complex libraries that either have lots of CDNAs in them or lots of antisense bit in them. In fact, you can go to the catalogue and buy these. It is very hard to assess the risk of a very complex library put into a retroviral vector of any sort. We don't know what is in there. What about andenovirus vectors? They are highly recombinogenic. Vector stocks that are supposed to contain only defective vectors may contain replicating virus.

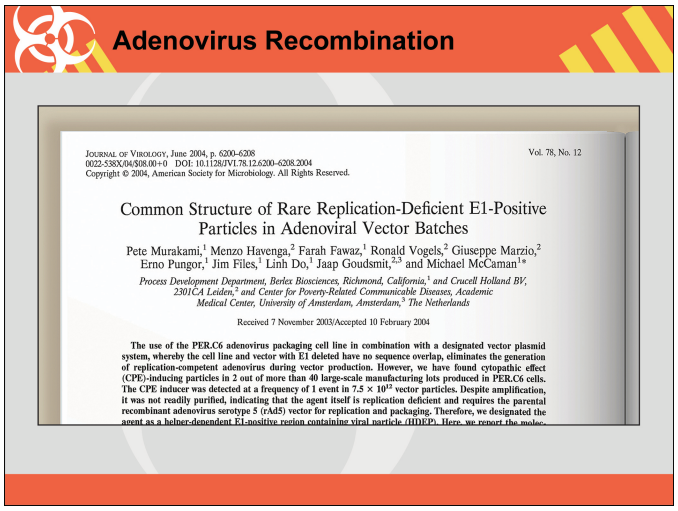


One of the things that is not a consideration ordinarily with retroviral vectors is that lab workers can be infected with adenovirus and that experimentalist can bring a replicating virus to the experiment.

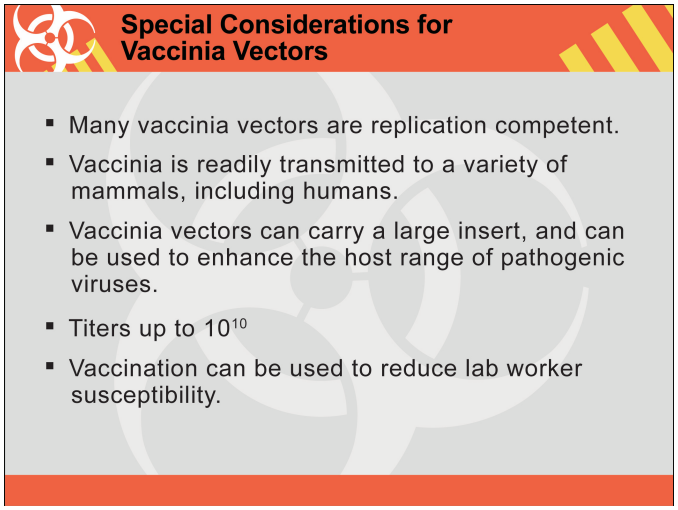
Finally, as I mentioned briefly earlier, this is a system in which people have deliberately made vectors with extended host range. Again, in terms of thinking about why these are highly recombinogenic, the titer on these viruses is very high. A very high titer stock of a retrovirus is ten to the seventh or ten to the eighth per mil. With adeno, ten to the twelfth is common. At ten to the twelfth, extraordinarily small volumes of fluid contain a lot of virus.



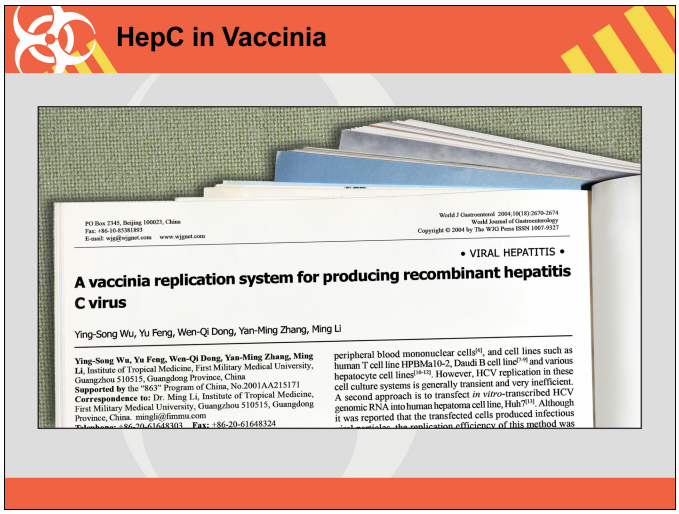
Here is, again, a publication showing, in fact, that recombination is a frequent problem. How about vaccine? In many cases, the vector itself is deliberately made replication competent. It is transmitted not just to humans, but also to a variety of mammals. The vaccinia vectors can carry a large insert, and this can be used to enhance the host range of a pathogenic virus. I will show you a particularly ugly example of that in a minute. Titers are not as high as adeno, but they are still high.



Here we have the advantage that there is vaccination that can reduce lab worker susceptibility. I strongly recommend that anybody who is contemplating working with vaccinia or supervising someone working with vaccinia, take advantage of vaccination.



So, here is what strikes me as an extraordinarily bad idea. This is one that I don't think our IBC would have allowed. People put a replication competent, Hepatitis C virus into a replication competent vaccinia. That, of course, allows Hepatitis C to have the host range of vaccinia. And they did it specifically because it would make it easier for them to infect certain cultured cells with Hepatitis C.



We are now going to talk a little bit about how to protect the workers. We have talked about what you should worry about, and of course, if you are going to talk about physical protection, it is going to have to match the risk. So, what is the expected route of infection?

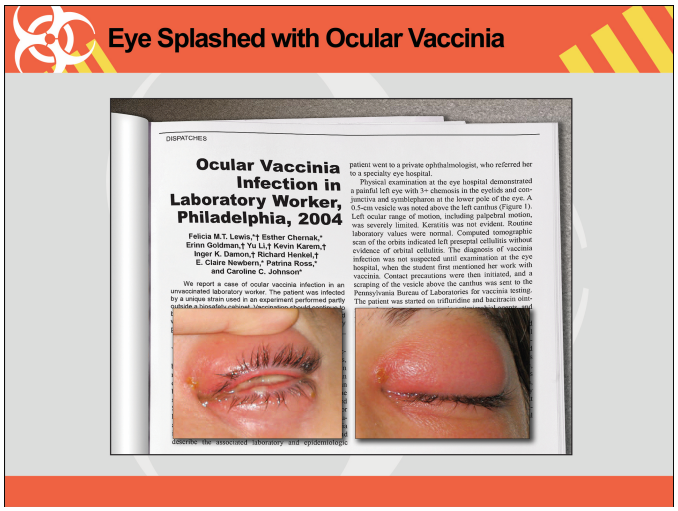
Before we do this, we are going to go through a few photos I pulled off the Web a couple of years ago and see how people deal with this and, in fact, how they deal with it badly. Unfortunately, these are not isolated examples.

This is from the avian flu epidemic a few years ago. What we see here is that these men have been out chasing the birds, but the eye protection does not work on the forehead. And almost certainly what happened here, since they have these men suited up, is that they have been running around chasing the birds. When you wear this kind of very tightly fitting eye protection, you perspire and your glasses become foggy. You can't see and so, you put your glasses up on your forehead. Well, that doesn't work.

Here we have what happens after you put your glasses up. You pull your mask down and you rub your eyes with your bare hand. Again, these men were given, presumably, the right equipment with the possible exception that they should have had different eye protection. Obviously, if they were given proper instruction, they paid no attention. Human error is part of the problem.

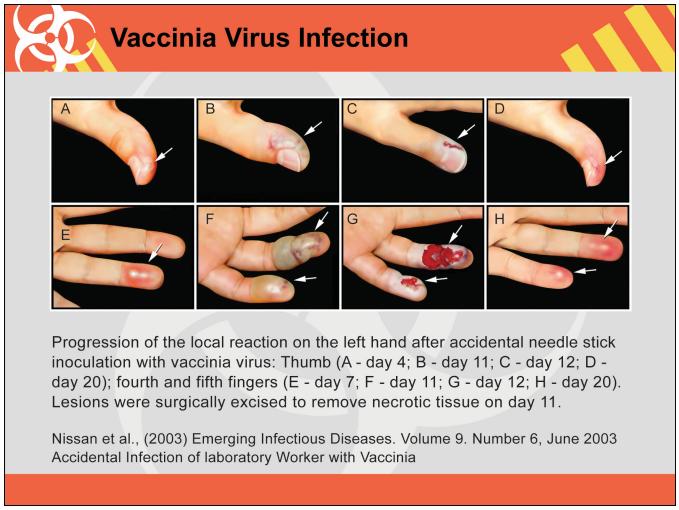
Here we have a lab worker working with what are presumably infected eggs. She has on a mask, but there is no eye protection. There is no hand protection, and there are no shoes. Now, these were taken outside of the U.S., obviously, but that is not to say that people in the U.S. don't make mistakes.

This is an ocular vaccinia infection. That is the eye after it got splashed with vaccinia, and that is what it looks like after you pry it open. It is a bad plan. Was she wearing eye protection? You get one guess: of course not. Nor was she vaccinated. Here is a case where vaccination would help.



People should think about what the consequences of their actions could be, particularly if something goes wrong or something gets loose, and consider how to deal with the situation before it happens, not after.

Needle sticks. Absolutely guaranteed to cause trouble. Here is a needle stick in the thumb with vaccinia. But this person managed to also stick two other fingers. That tells you automatically that they did not stop after they stuck themselves the first time, but they continued to fool around with the needle and got stuck a second time.

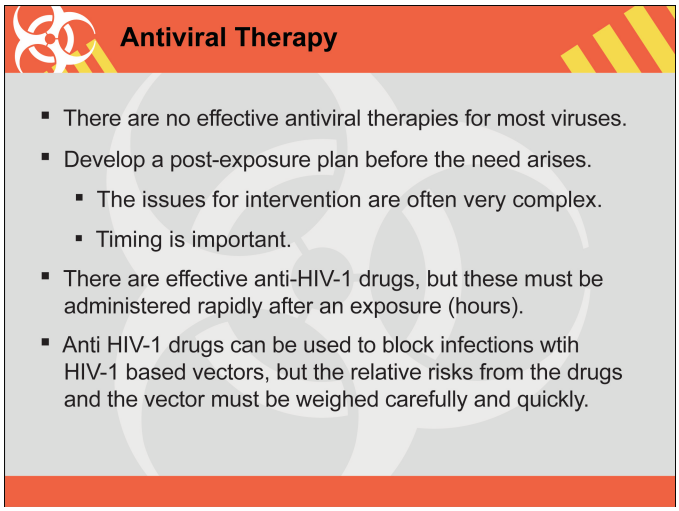


You cannot get the human error out of the equation. The trick is to try and figure out a protocol for the person and unless they do something really foolish like this, you reduce the chance that something is going to cause trouble.

Sometimes there are failures, and the question is, what can we do afterward? Unfortunately, in many cases, there are no good antiviral

therapies. You get hit with adeno, but it is pretty much too late. Vaccination – also too late.

Nonetheless, there should be a post-exposure plan in place before the need arises. The issues for intervention are often very complex and the timing is important. For example, there are effective anti-HIV drugs, but these must be administered rapidly after an exposure. We are not even quite sure how rapidly, but it is hours, not days.

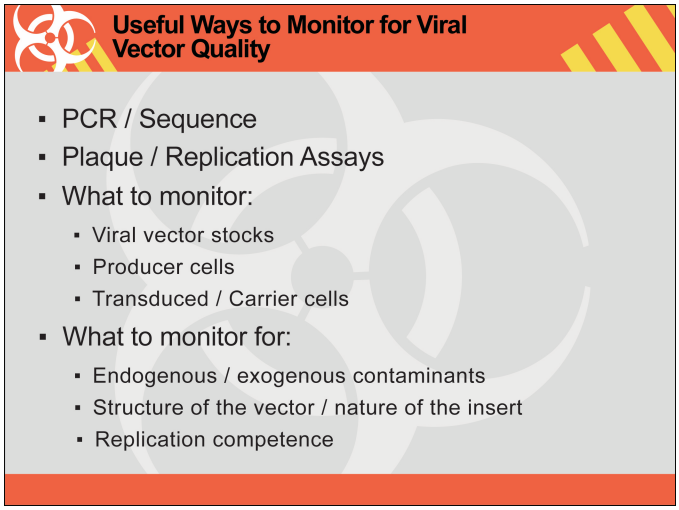


The relative risk from the infection has to be weighed against the relative risk for the drugs. Everything has to be properly prepared and in place well before the accident happens. The healthcare worker has to be in place, the drugs have to be accessible, and there has to be a way to bring the person who had the accident, the healthcare provider, and the drugs together in a timely way. That plan has got to be in place. Experiments sometimes don't work as planned, and bad things happen.

Another incredibly important aspect is to make sure there is proper quality control. We are now in a position where we are much more able to determine what we are working with and how potentially dangerous it is, and to make sure that the person has the tools and reagents they think they do. PCR sequences are probably the obvious and simplest way to do that. There are plaque and replication assays, and there are

better ones being developed that can distinguish the presence of nucleic acid, for example, from the presence of true replicating virus.

Obviously, we want to monitor viral vector stocks, producer cells, transduce and carrier cells. If something has been passed through an animal, then we want to look for either endogenous or exogenous contaminants. We need to understand the nature and the structure of the insert. And, of course, because replication competence is one of the serious issues, we want to make sure that if we have been told it is a defective virus stock, that it is a defective virus stock.

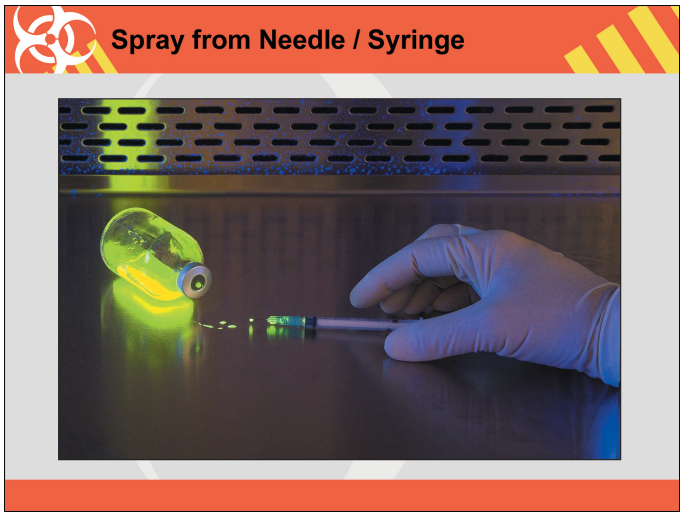


So how do you set up a safe system? You don't want anyone to go in full bore with an agent that is potentially dangerous. We like to have a test run with fluorescent markers, which can track materials prior to using live agents. They are easily tracked by UV light, and it sometimes is a real epiphany to the person developing the procedure when the whole front of his or her lab coat is splattered with fluorescent spots. The markers we usually favor are riboflavin and fluorescein.

This is fluorescein. If there is a spill, it is obvious and very small volumes can be detected, not as small, of course, as the volume that is important if you have a red-hot adeno stock, but still small volumes. And you have to pay attention, because fluorescein will photo bleach if it is in small amounts.

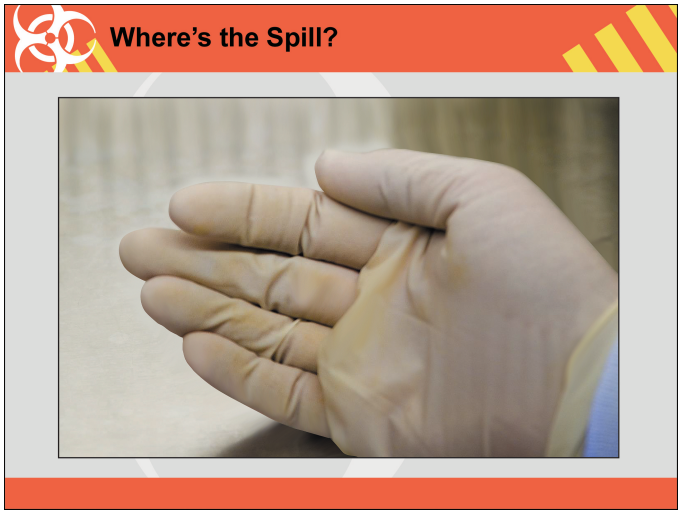
This is me on a camping trip up in Alaska. I was making pancake batter in a soda bottle. The good news is it was pancake batter and not something more serious, because after I shook the bottle up and opened it, I got sprayed rather thoroughly all over my shirt, hat and face with the contents of the bottle.

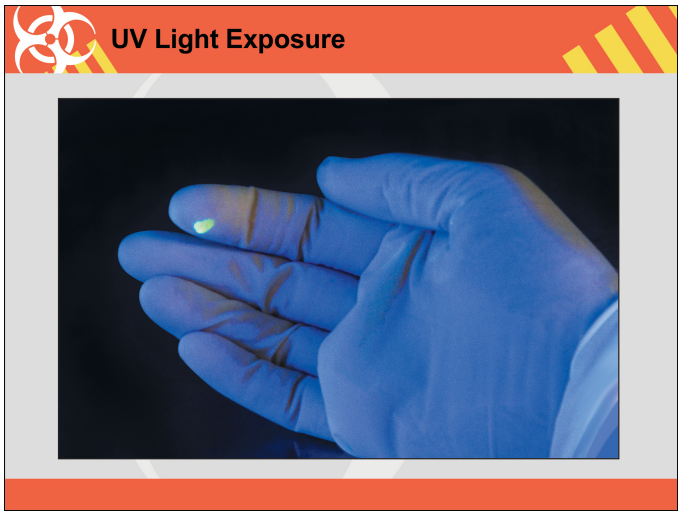
It was, again, a reminder that if you have any reason to suspect the contents are under pressure or if there is any way that the contents can be under pressure, you need to be careful. This particularly pertains to taking things out of bottles – sealed bottles with syringes – because you can get a spray. If you are dealing with a high titer stock or a potent toxin, small volumes matter.



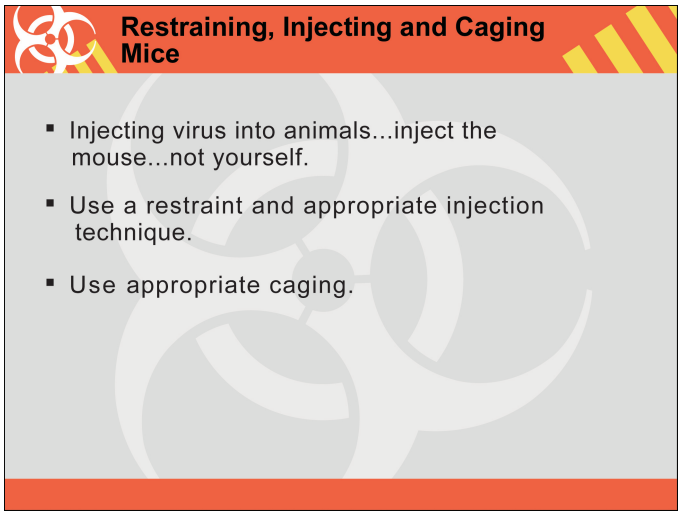
We also have the issue that if you take things out of vials, the lip of a vial and sometimes the cap can be contaminated. If there is a question, get a clean tube. Is your hand contaminated? Yet bet.

There is the final message for those who think that wearing gloves is safe. Change your gloves. Gloves get contaminated. If you touch something that then touches something else, pretty soon the stuff is not where it belongs.

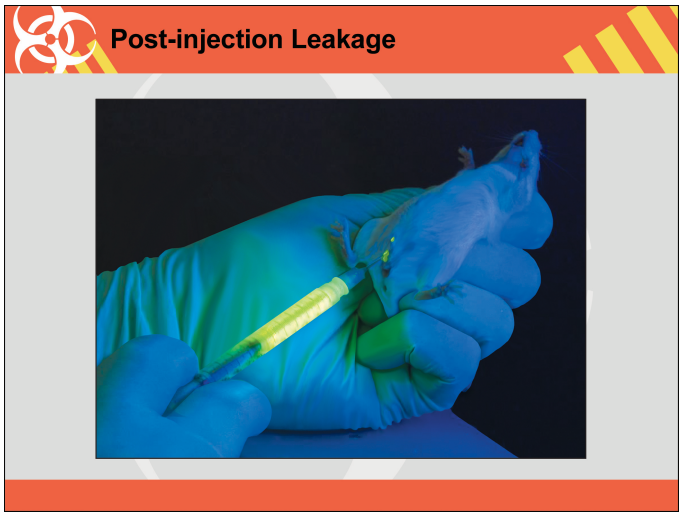




To finish up the issue of dealing with animals, and particular sharps, the trick is to inject the animal with the needle, not the person. Restrain and appropriate injection technique and appropriate caging are essential. Even if the injection is done carefully, there can be leakage. Also, if a mouse has something interesting on its hide, anyone who handles that mouse could come in contact with whatever it is.



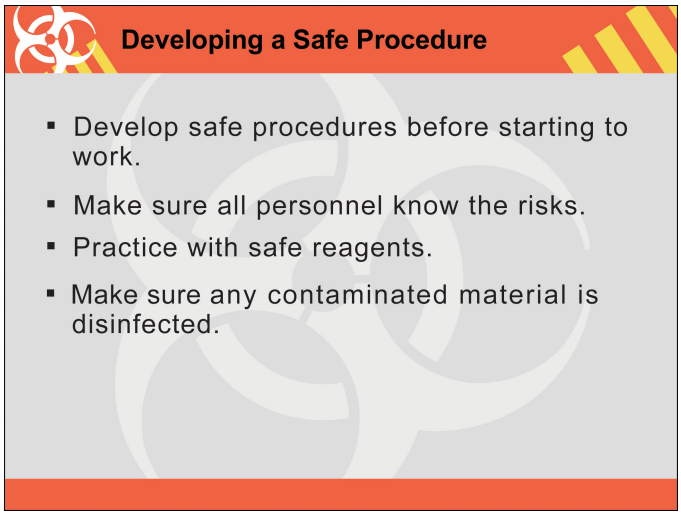
This is a technique that we don't like because the needle is pointed in the general direction of the hand. This should be avoided if possible.



Restraint is much better. In this way, when things spray, which of course can happen when you pull the needle out or if you don't hit the tail vein exactly, it is not necessarily as bad.

So, how do we go about developing safe procedures? The safe procedures should be developed before the work begins. That should be axiomatic. All the people, and this particularly applies to people such as animal handlers, need to know the risk. They do not need to necessarily understand the details of the experiment, but need to know what it is about the experiment that could hurt them.

I strongly recommend practice runs with safe reagents. We do large volume virus growth, and we always recommend that the people practice pumping fluorescein before they start pumping virus, particularly if they are using high-pressure pumps. Make sure that any contaminated material is disinfected, and by this, it is incredibly important to have the disinfection protocol manage the susceptibility of whatever is being disinfected. Not all viruses, not all pathogens, can be disinfected in the same way.



The ultimate message is that it is humans and human error that really are the most dangerous part of research. I've never seen a serious lab accident in which the person doing the experiment had a clear, careful, well thought out protocol and followed that protocol religiously. The accidents always involve people who either did not have the right protocol, which is usually not the case, or they did not follow the protocol and got themselves contaminated.

I am going to quit now and take questions. I want to thank Bruce Crise and Joe Kozlovac, whom I have worked with a long time on biosafety issues.

Question: The point that you made about the commercially available lenti vectors having misleading information – could you elaborate on what the misleading part is?

Dr. Hughes: One of the things that certainly has bothered me is the claim that because you've divided the genome into pieces, you can't recombine the pieces. And, again, this often has to do with whether or not there is overlap. It does reduce the rate of recombination to have divided the genome and its complimenting parts into more than two pieces, but it doesn't go to zero. To say that the virus is safe because it is in pieces is not accurate. The primary reason that the modern viruses are safe is that they are VSVG complimented and they have a dilution envelope, so there's no way to reconstitute the envelope. But the rest of the genome can recombine, and the emphasis, in that regard, in my opinion, is wrong.

Question: We have a lot of scientists who are focused on their science, and, unfortunately, not focused enough to really plan their experiments. How do we get that culture of being sure that risk assessment is done and done realistically

Dr. Hughes: I could not agree with you more. I am reminded of an adage from my childhood: There's not time to do it right, but there's always time to do it twice. We have had, obviously, similar discussions with some of our colleagues. My answer to them is, you really should not be doing experiments that you have not thought through very carefully, because there will be variables that you haven't taken into account. And part of safety is simply good microbiology.

Scientists are people. And, unfortunately, that is why we have to say that the human error is part of the equation. I think it's an ongoing education process. It is perhaps the biggest part, and the most difficult.

Comment: One thing that our university has done, which has helped a lot, is that they've helped scientists through the risk assessment. We have helped researchers plan out parts of the risk assessment from the biosafety end of things. And that helps the scientific community in that if you, as a biosafety officer, can help plan out the risk assessment a little

bit for the researcher, it helps. Instead of telling researchers what to do, they are helping them see the light, in that if you do this from the beginning you won't have to go back and do it a second time. And most scientists don't want that second time around, because, that's wasting time and effort, and money.

Dr. Hughes: There is an aspect of this that is very important. If there are adversarial relations between the researchers and the regulators, you are not going to station an IBC policeman in the lab, even a BSL-3. And so, to some degree, this has to be with the cooperation of the researcher.

And so to what extent – whatever extent it's possible to have a sort of a pedagogic relationship where you can help educate, teach the people both the risks to the science and the risks to the human – that is very much a good thing. And it is the IBC that should, in my opinion, be more than simply an enforcement agency. It should also be one for education.

Permission granted to reprint a portion of:
Hughes, S. H., & Cruise, B (2007). Using Viral Vectors in Animal Research. In J. Y. Richmond (Ed.), Anthology of Biosafety X: Animal Biosafety, pages 145-166. Mundelein, IL: American Biological Safety Association (ABSA).

3

Practicing Safe Science

Laboratory Techniques for Biohazard Control

Laboratory techniques that minimize potential exposures to biohazards are essential for all laboratory studies involving biological materials. The level of containment required for safe and secure conduct of the study should be determined after a careful risk assessment of the research protocol. This is an essential starting point whether the hazard to be contained is a select agent or another infectious agent, a genetically modified organism or plant, or human or animal tissue or body fluids. The risk assessment drives the selection of biosafety practices required to protect the research staff, the product, and the environment.

Setting Containment Levels for a Laboratory Practice – Infectious Dose Consideration

A starting point in the risk assessment for a protocol involving infectious agents is consideration of the infectious dose. A literature search will generally provide some data about the concentration of an infectious agent required to initiate infection. However, agents may be transmissible by one or more means of exposure (parenteral, mucous membrane, cutaneous) and the infectious dose for each will vary. In addition, the agent strain that the laboratory plans to use may not have the same virulence characteristics as the strain used in the publication. However, tempered with the understanding that there is probably a range of infectious dose, and a range of individual susceptibility, knowing the infectious dose is the first step of a responsible risk assessment.

In addition to knowing the infectious dose, the next step is equally important – determining how the infectious dose relates to the concentration of organisms handled in the experiment. There are also questions that should be asked of the investigators that affect the risk

assessment. What is the titer of the agent stocks used in the laboratory? Are they growing large volumes? Are the investigators going to prepare concentrated preparations of the agent? Do they plan to purify the virus by banding in ultracentrifugation? Is the agent going to be used in an animal model? Passage through animals can change the nature of the agent under study. Relating the infectious dose to the risk of exposure for each step of the given experiment is an important exercise. Have the researchers evaluate, for instance, whether a drop of culture, an aerosol, or a needle stick could contain an infectious dose? How long is the microorganism likely to retain infectivity, if the work surface is not appropriately disinfected after use? Is transmission from fomites a potential route of exposure?

Directly involving principal investigators in the risk assessment process is invaluable for establishment of sound laboratory practices. It is recommended that university biosafety programs provide principal investigators with a template to help guide them through the risk assessment process. The University of Pittsburgh developed such a template following discussions with investigators submitting an Institutional Animal Care and Use (IACUC) protocol since all of the hazardous materials, including biohazards, must be carefully evaluated prior to use in an animal model. A well-designed form that leads to a proper risk assessment will streamline the approval process. Biosafety programs that provide the ground-level tools for this process set an appropriate framework for the collaborative effort that is required to ensure prudent biosafety practices during all phases of the research.

Safety Culture

Safety culture is the attitudes, beliefs and perceptions shared by natural groups as defining norms and values, which determine how they act and react in relation to risks and risk control systems.[16] Advancing a culture of research safety and a safety-conscious work environment requires executive commitment, support of senior scientists, and proactive involvement of environmental health and safety and laboratory staff. Principal investigators must serve a leadership role in creating and sustaining a safe and compliant research environment. They must set

[16] Hale, AR (2000). Culture's confusions. *Safety Science*, Volume 34, No 1-3, pages 1-14.

and maintain high personal safety standards and make clear the expectation that all laboratory members will habitually incorporate the fundamental good rules of safety into their daily work routines.

Role of Biosafety Professionals in Promoting the Safety Culture

The more familiar a biosafety professional is with a laboratory's operations, the better chance he or she will have to be a positive force for change in those operations. This includes becoming a known and positive resource for principal investigators, since without their buy-in to the biosafety program, little change will occur. Principal investigators should consider biosafety professionals as their partners in helping to ensure that students and staff are aware of the hazards associated with biological materials; are proficient in techniques to prevent exposures; are trained to safely conduct new protocols that may be introduced into the laboratory; and are aware of and comply with all health, safety and environmental requirements, standards and operational practices of the institution.

Biosafety professionals should collaborate with the principal investigators and laboratory staff with the goal of working as a team. One example that demonstrates the value of a collaborative partnership occurred at Harvard University. When the HIV virus was first brought into a laboratory on the campus of Harvard, because the biosafety professional knew the laboratory staff, she was able to assure them that the first step in determining appropriate containment practices would be observation of their standard procedures. She was welcomed into the laboratory where she literally sat quietly on a stool in the corner for an entire day. That enabled her to make recommendations relevant to that laboratory's operations – for example, eliminating the use of needles and syringes to remove virus from test tubes, reducing their risk of exposure. Without an established working relationship with researchers doing the most hazardous work in a program, a biosafety professional cannot hope to make recommendations that will be adopted or even considered. As one biosafety professional commented at the Safety by Design Symposium, "We have the eyes to see what the researchers may not see as a potential exposure incident." Establishing this level of collaboration and trust assures researchers that the interventions will be reasonable,

evidence-based, and appropriate to the level of risk. This approach also builds the appropriate groundwork to assure that spills and near-misses will be reported.

Role of the Institutional Biosafety Committee

The Institutional Biosafety Committee (IBC) provides value support to the biosafety program. Whenever a biosafety professional and researcher disagree about containment for a given procedure, it is important to refer the question to the IBC. IBC review will work to resolve the disagreement by either 1) supporting the requirement for a standard biosafety practice, or 2) by supporting an exception, which for example, would be based on factors such as the data confirming an agent's attenuation, or its inactivation by the chemicals used in the experiment. Regardless of the outcome, the credibility of the biosafety program is enhanced when its mandates are open to review and input.

Authority of the Biosafety Professional and Empowerment and Responsibility of the Research Staff

"Years ago working as a technician, my principal investigator said to me, 'If I ever ask you to do something and you don't know exactly what you are to do, don't do it; come talk to me.' This is a message that must be communicated in the scientific community. Supervisory staff must provide clear instruction that goes beyond mandated training. If there is a concern about safety practices, lab members are responsible for questioning the practice, and PIs are required to respond appropriately."

– Rosamond A. Rutledge-Burns, MS

Biosafety officers must have the support of senior management and be granted the authority necessary to effectively carry out their role. Whether the biosafety program is in private industry, academia, or the federal government, support of it must come from the highest level of management. It is equally important that senior management support the staff that actually performs the operations of scientific studies. Technicians and animal care

staff, for example, should be empowered to question procedures or situations when there is apprehension or uncertainly and to report their concerns. It must be communicated and understood within the scientific community that safety is a team effort. If anyone has a concern about safety practices, it is their responsibility to make known their apprehension and ask questions, and they must feel comfortable doing so without fear of retribution. It is the responsibility of the principal investigators to be attentive to safety issues affecting their laboratories and to respond appropriately to resolve those issues collaboratively with the environmental health and safety professionals. Principal investigators must also ensure that the roles and responsibilities of each individual within the laboratory are unambiguous with respect to adherence to good health and safety practices and compliance with applicable health and safety regulations. Accountability in safety matters must be clear.

Addressing Competence in Standard Microbiological Procedures

The crossover between disciplines is exciting scientifically, but physical scientists do not necessarily have the experience required for a complete understanding of good microbiological techniques. A staff without a strong microbiological background may not be competent to assess, for example, the potential repercussions for pathogenicity if changes in tropism occur. Institutional support for mentoring programs may be required when there are gaps in relevant microbiological experience for the conduct of biohazardous experiments.

Focus on Biosafety Level 2

In general, the proliferation of Biosafety Level 3 (BSL-3) and Level 4 (BSL-4) laboratories has resulted in the appropriate regulatory framework that limits access to the pathogen proposed for study in these laboratories to staff who are knowledgeable about the pathogen, experienced with the procedures that will be performed, and diligent in following safety requirements including the donning and doffing of personal protective equipment (PPE). Due to the hazards routinely handled in BSL-3 and -4 laboratories, there is regulatory capacity to

restrict access to those who are non-compliant. This provides scientists, administrators, and biosafety officers more control over laboratory operations at BSL-3 and -4 levels than at Biosafety Level 2 (BSL-2).

With less regulatory oversight of BSL-2 research, how does a biosafety profession foster the development of a safety culture that would consistently implement BLS-2 practices, in particular, the use of PPE? How does one discharge the responsibility for staff safety if, for instance, enforcement of a basic requirement to wear laboratory coats and gloves antagonizes the staff? One of the most important steps for biosafety professions is to set a positive example. This would include wearing the PPE required for laboratory operations during laboratory audits or observation exercises. Also, benchmarking and using reported laboratory incidents as learning experiences may bolster compliance with wearing PPE. Guides for task-specific PPE posted in the work area along with the PPE may encourage PPE use.

Consistent enforcement to PPE requirements is important. Biosafety professionals must ensure that when a door placard lists certain PPE required for entrance, all those entering are in compliance with the stated requirements.

Having support of the principal investigators in enforcing PPE and all other health and safety requirements is important. They must promote the principle that safety is a core value in the conduct of science. For example, at the University of Pittsburg, during the summer months some laboratory staff in BSL-2 tissue culture labs appeared in shorts and flip-flops. The principal investigator had established that laboratory coats and closed-toe shoes were required; this basic PPE was not optional in his laboratory. The policy is stated during initial interviews with staff, and reinforced when required. The staff explained that they wore shorts because they bike to work and were not comfortable doing that in jeans. The principal investigator made no exceptions. Now, when staff arrives at the laboratory, they can shower and change into dedicated scrubs, lab coats, and shoes.

In the end, the goal is research safety, not just biological safety, since there are many other factors in the conduct of biological experiments. The PPE requirements should provide appropriate protection for all of the risks involved in a given experiment.

Poster illustrating task-specific PPE.

Laboratory practice at BSL-2 should focus on preventing parenteral exposures. There are some substitutions that can be particularly effective, for example, glass Pasteur pipets are used for aspiration of tissue culture media from cell lines, many of which must be handled at BSL-2. There are alternatives – sterile plastic Falcon aspiration pipets, P1000 or P100 pipet tips, or the Corning aspiration device or similar tools that allow controlled, aseptic aspiration. The Dana Farber Farber Cancer Institute IBC banned the use of glass Pasteurs for BSL-2 experiments. This eliminated the two to three exposure incidents per year that occurred from the use of glass Pasteurs. Staff were initially reluctant to relinquish this common practice, but the logic of removing pointed, sharp glass from BSL-2 experiments was obvious, and they complied.

In clinical settings, the dramatic reduction in needle sticks has resulted from the use of safety-engineered needles and syringes. Some of these devices are unwieldy in the research setting, since some of the designs are too bulky to be inserted into Eppendorf or other test tubes. Evaluating other options can result in effective substitutions.

Substitution of "safer" products can reduce other types of parenteral exposures. Cuts from glass Dounce homogenizers or haemocytometer cover slips can be eliminated with plastic substitutes; inexpensive plastic collars are available to protect fingers while snapping glass lyophilizer

tubes open. Metal decrimping tools can safely remove the metal seal around a septum vial, allowing removal of the rubber stopper and insertion of a pipet instead of a needle. Needles can be eliminated from DNA shearing; a closed, focused sonicator such as the Covaris will provide more reproducible results. Cut-resistant gloves can be worn under exam gloves to reduce cuts from microtome or cryostat blades, and small artist paint brushes to remove tissue sections from the blade are useful additions to a histology procedures, particularly when staff are learning procedures. Use of disposable safety scalpels, or blade removers for reusable scalpels, minimizes exposure incidents. During necropsy, using blunt forceps and scissors as much as possible reduces the risk of puncture wounds.

Many incidents could be eliminated with simple steps such as using a test tube rack to hold items or the elimination of razor blades. To prevent mucous membrane exposures, as well as glove or environmental contamination, gauze or safety de-cappers should be used to remove the stoppers from Vacutainer tubes. A challenge for biosafety professionals, especially in a large academic institution, is to get the ordering information for products that will minimize risk to the individual researchers.

Biosafety Level 2 Laboratory Design

Many aspects of BSL-2 design impact laboratory practice. When scientists and environmental health and safety professionals are not involved in the design phase of construction, designs without the required features for safe laboratory operations can result. For example, having desks with computers at the end of the laboratory bench increases the likelihood that staff will eat and drink in the lab because it is common practice to eat and drink at one's desk. Adequate space for chemical storage with appropriate segregation of acids and bases, and flammable storage in compliance with National Fire Prevention Association (NFPA) requirements minimizes audit and compliance issues. Because new or renovated facilities will be certified and tested for compliance with BSL-2, -3, or -4 requirements or certified for pharma purposes, the commissioning agent should also be involved in the design phase.

Many new buildings under construction have large open laboratories, which present new challenges. Administrators often issue space assignments. Without the proper input from the biosafety professional, adjacent benches could have different containment levels. For example, there would be significant contamination issues when an investigator working with yeast shares space with an investigator working with human stem cells. It is important that the scientists and biosafety professionals work with administrators to allocate the laboratory space.

The popularity of the open laboratory concept is due in part to the thought that the concept saves energy. In open laboratories, it is important that scientists and environmental health and safety staff work together to develop plans for effective and safe use of the shared space and the safe handling of biohazards, chemicals, and radioisotopes within the space. Also, in the open laboratory concept there are additional challenges for maintaining the space and for the potential for cross-contamination.

Improving Laboratory Practice with Standard Operating Procedures

In the US, laboratory practice is generally managed with standard operating procedures (SOP), and a training requirement that staff sign a statement declaring that they have read and understand the SOPs. This, however, does not necessarily result in uniform adherence to the SOP. A written SOP does not replace good communication of the required procedures. It is important that time be allotted for the laboratory staff to recreate the activity described in the SOP in order to provide input on any steps requiring clarification. Involving the laboratory staff in testing SOPs for everyday laboratory operations improves compliance and reinforces the collaborative approach to developing good biosafety practices. Biosafety professionals should work with principal investigators to ensure that all necessary SOPs are in place and current.

When promoting laboratory practices that require additional time and effort on the part of the research staff, the first question often asked is "where is the data supporting this?" There is a pressing need for additional applied biosafety research to evaluate, for example, the containment of the new types of instrumentation found in laboratories

today. Robotic pipettors, cell harvesters, plate readers with built-in sonication steps – are these sources of aerosols? Biosafety professionals need these answers to develop appropriate BSL-2 laboratory practices and be able to provide the research staff with the reasoning and evidence supporting required laboratory safety practices.

3

Practicing Safe Science

Care and Use of Laboratory Animals

There is an essential role that research and education involving live animals has in the advancement of biological and medical knowledge. Animals used in biomedical research and education should receive the best possible care and be treated with respect. The Animal Care and Use Committee (ACUC) is established to oversee an institute's animal program, facilities, and procedures and to advise the Scientific Director on all matters of animal care and use at the institute. Accordingly, the ACUC is responsible for ensuring that the institute's animal care and use program is operating in accordance with applicable guidelines and regulations. Furthermore, the committee serves as a forum for discussion and decision-making regarding issues impacting on the sound application of animal resources to meet institute scientific objectives.

While providing the very best standards in animal welfare and care researchers must also ensure that they are working with the correct biosafety standards to ensure protection of themselves, the community, and the environment. These needs must be carefully balanced with the needs of the animal to ensure all requirements of animal welfare and biosafety are met in every study. Additional challenges occur when using non-traditional laboratory animals such as chickens, pigs, goats, and various wildlife species. The degree of biocontainment necessary is heavily predicated upon a thorough site and activity-specific risk assessment and should be based upon performance-based guidelines.

Risk Assessment

Use of animals in research varies to a large degree. Many different species are used as animal models, and an array of microbial agents,

chemicals, toxins, and pharmaceutical products are used in a range of experiments that require the use of animal models. Therefore, it is not possible to be prescriptive in the type of biocontainment needed for any one study. A thorough and complete risk assessment that addresses both facility capabilities and specific activities being performed must be done before any research is started. In all cases, the appropriate experts should be consulted and their recommendations documented in order to justify the biosafety and biocontainment facility features, protective equipment, and practices used to conduct the work. The individuals involved with the assessment and conduct of the work should be experienced with the animal species or arthropod being used and have knowledge of the type of study being done. For further information on how to conduct a risk assessment, one should consult the USDA and CDC informational training documents, available from USDA or CDC. Information to be evaluated during the risk assessment include:

- Virulence and pathogenicity
- Route of transmission/Infection
- Agent stability - biological decay
- Infectious dose
- Quantity and/or concentration
- Endemic or foreign to the region
- Availability of data on morbidity and mortality
- Availability of effective prophylaxis, treatment or vaccines
- Pathogen shedding and transmission patterns in relevant species
- Existence of geographic control or eradication programs for the disease
- Animal species (large or small)
- Specific procedures/activities being performed
- Pathogen host range
- Existence of geographic surveillance testing

Animal Selection and Quarantine

Before bringing any new animals into a study careful selection of the animals and their health status needs to occur. Of great concern to the researcher related to public health or agricultural research is the

introduction of unwanted pathogens into the research environment. One common mechanism for the introduction of new organisms to a facility, either laboratory animal facility or research barn, is through the introduction of new animals. Prior to accepting new animals into a facility, the health status of the animals for important diseases of consequence or those that could negatively impact the experiment should be determined. The type of screening assay performed is dependent on the pathogen of interest and can include swabs, serology or running tests to ensure that the animals lack any diseases or organisms with the potential to disrupt the experiment or production system. That is why working with a subject expert, typically a veterinarian, to determine what pathogens the animals from source farm/vendor should be tested for can minimize the risk of cross-contamination of an experiment or research animal facility. (Kozlovac 2012).

When possible, animals should be selected for a calm demeanor and high health status. There may also be a need for separation of animals by health status, especially for the breeding colonies, where the highest possible health status should be maintained. Selection of animals based upon health status requires the availability of standard and special diagnostic panels by species, in order to screen out adventitious agents and latent infections that could compromise the health of the breeding colony or adversely affect the study. For some species, including many of the traditional animal models, such as mice, greater choices in specific-pathogen-free animals are now available to enhance science and biosafety. Animals of unknown or suspect health status entering a facility should be quarantined, tested and monitored before being allowed to enter the main colony or being used for a study. Animals should also be allowed to become acclimated to the local environment, food, caging, and animal handlers.

Care and Handling of Infected Animals

All applicable regulations and guidelines must be followed when using animals in research.

Different species of animals are typically housed in different rooms to accommodate the individual species needs regarding their

environmental needs and prevent cross contamination of adventitious pathogens and interspecies conflict. With the advent of individually ventilated cage systems, certain situations may allow multiples species to be housed in the same room. Enrichment of the animals' environment by a variety of different methods must be considered and provided if possible.

A greater number of different animal species and arthropods are being used for research on a wide variety of topics, which has necessitated careful review of protocols by the ACUC and Institutional Biosafety Committees (IBC).

Increases in diagnostic abilities and greater sensitivity in tests has now allowed for greater monitoring of animals for a wider variety of pathogens during the study. The use of telemetry for health monitoring and other means have also allowed for monitoring animal health remotely, for example cameras and audio capability in the room, to see and hear the animals without having to enter the room. Telemetry allows for more and better quality data, less stress to animals, increase safety for personnel, and can help to identify early endpoints.

Manipulation of the immune systems of animals has created greater difficulties in maintaining barrier protections of the animals from external pathogens. Many animals are completely immunocompromised allowing them to be easily infected by many common microbial agents, which requires special housing/handling and careful attention to detail by animal care and research staff to prevent the inadvertent introduction of pathogens. To prevent the introduction of disease organisms and reduce the potential from cross contamination between animal rooms and facilities, many researchers, animal facility managers and veterinarians will utilize procedural controls including shower-in, shower-out practices, use of dedicated clothes and boots washed and cleaned in the animal facility, specific animal room entry order, and in some cases, air filtration on supply air, all to minimize the potential for introduction of arthropod vectors, fomites as well as microorganisms.

Animal welfare guidelines require greater and more frequent observation of animals for general health, discomfort, pain, and distress to ensure they are being used humanely. Clinical scoring sheets should be used to record the animals' condition and the need for medical or endpoint intervention. If necessary, animals must be treated, euthanized

or removed from the study. Animal welfare guidelines require that pain or distress must be relieved in animals, and if not, a very thorough and scientifically sound justification must be provided for why analgesics, anesthetics, or sedatives cannot be administered. Risk assessments should take into consideration that a consequence of this appropriate emphasis on the alleviation of pain and distress is that it can potentially increase the handling of animals, which may increase the level of risk in a biocontainment environment. Animal welfare guidelines require regular assessment of animal health, and death is no longer considered a default single endpoint for animals. Frequent monitoring and thorough clinical assessment potentially require greater animal handling and manipulation during a study.

Advances in inhalant and injectable anesthetics have allowed greater biosafety by providing a means for sedating or immobilizing animals that are infected with highly dangerous pathogens. To protect the animal care worker, personal protective equipment, selected based upon the experiment-specific risk assessment, should be worn at all times when handling animals. This may include a variety of hand and arm protection that is bite, scratch, and puncture resistant; face splash protection; respiratory protection; body protection such as gowns and coveralls; foot protection such as steel-toed boots; and hearing protection. Great care should be taken with sharps of all kinds in the vivarium. These come in the form of needles, scissors, pasture pipettes, scalpels, knives, saws, fangs, teeth, claws, and sharp edges on cages, to name a few. As many of these sharps as possible should be eliminated from use in the vivarium. If still required, engineered sharps, such as integral needle safety systems, disposable scalpels, and blunt-tipped scissors, should be used when possible. A veterinarian can blunt sharp canine teeth on animals if it is necessary to work in or around the mouth of an animal in a high-hazard environment.

Necropsy

Conducting necropsies on animals is one of the highest risk operations during an animal study. Animals must be removed from their containment caging, euthanatized, and directly manipulated by the prosector, thereby potentially exposing the prosector to any agent, toxin,

or chemical that the animal may harbor. A variety of sharps and cutting instruments must be used, particularly when performing necropsies on large animals. Teeth, the edges of cut bones, and implanted instruments (e.g., telemetry devices) also provide additional sharps that must be accounted for. In addition, contaminated aerosols and droplets can be generated during a normal necropsy or especially in the case of careless manipulation of the carcass or individual organs. Additional risks include: a) exposure to pathogens from contaminated surfaces; b) decreased dexterity, vision, and hearing; c) fatigue, dehydration, anxiety, and stress; d) slips, trips, and falls; e) chemical and electrical hazards hazards; and f) removal of fixed tissue containers from the biocontainment necropsy area.

"The conduct of necropsies is one of the most risky procedures done in biocontainment labs. Yet, the risk of injury associated with biocontainment necropsy has been granted little direct attention in biosafety publications."

– Keith E. Steele, DVM, Ph.D.

For complex necropsies personnel should be formally trained and mentored, experienced, confident, reliable, and healthy. Complex necropsies should be conducted in teams with the minimum number of people for the task, which is carefully planned and practiced beforehand using uncontaminated animals as controls whenever possible.

In 2006, the National Association of State Public Health Veterinarians published excellent guidelines and recommendations for precautions to take during a necropsy. These precautions include:

1. Aerosol exposure
    a. Minimize procedures that create aerosols during necropsy, such as opening gas distended pouches, excessive bone sawing, spraying water, etc.
    b. Use a downdraft table or other engineering control, if appropriate for the species and procedure, to draw infectious aerosols and anesthetics/volatiles away from the face when possible. Connection to the building exhaust or filtration of air may be necessary.

c. Use an appropriate respirator (e.g., N99 mask, Powered-Air-Purifying-Respirator, Half or Full Face Respirator with High Efficiency Particulate Air [HEPA] filters) selected as part of the risk assessment during the conduct of aerosol generating procedures of the necropsy, in which there is a potential for occupational exposure.
d. Use a ventilated enclosure (e.g., flexible film isolator, hanging curtains with directional airflow) for procedures known to create infectious aerosols and droplets such as cutting bone.

2. Percutaneous exposure via cuts or needle stick

   a. Use extreme caution when using knives and scissors when doing the necropsy to avoid injury.
   b. Use protective cut-resistant clothing, in particular gloves, which help minimize minor cuts and punctures to the skin.
   c. Use engineered safe sharps whenever possible such as retractable needles, sheathed scalpels, and blunt-tipped scissors.

3. Mucus membrane exposure

   Use face shields, goggles, face masks, or other personal protective equipment to cover as much of the mucus membranes (mouth, nose and eyes) as possible during procedures that have the potential to cause splashes of infectious material.

4. Oral exposure
   Use a face mask to cover the mouth and nose to ensure nothing enters the oral cavity. This will also protect from accidentally touching the mouth and nose with gloved hands that may carry infectious organisms.

Because of the difficulty in physically manipulating a large animal species, wearing a lot of personal protective equipment is challenging. Workers become overheated and may be tempted to remove the extra

layers of protective equipment, or protective equipment may get dislodged due to contact with other heavy equipment in the necropsy area, such as hoists, hanging chains, and cart handles. Due to the size and weight of the animal carcasses, heavy lift equipment and carts are often needed to elevate and move the carcasses. In many cases the whole carcass is too big to fit into a cart, destruction equipment charging head, freezer, or doorway and needs to be cut into smaller parts, adding further risk to the operation.

Carcass Disposal in the Vivarium

Disposal of carcasses in the vivarium can be simple or complex depending upon local regulations, available infrastructure, the species and number of animals being disposed of, as well as the nature of the infecting organism. In the case of small numbers of animals (e.g., rodents, chickens, quail, rabbits, fish, young stock), the biomass is not considerable and disposal is manageable. In most instances autoclaving is sufficient to sterilize the infected carcass of small species prior to final disposal in a municipal landfill or by burning in a licensed medical waste or pathological waste incinerator.

Large species or large numbers of carcasses can easily exceed the capacity of a standard-sized laboratory autoclave resulting in the need to temporarily store carcasses. Additional options include: burying, incineration, rendering, digesting or using a combination of steam and maceration. Check with your state and local regulatory agencies regarding specific disposal requirements and restrictions. If working in an ABSL-3 and above high containment vivarium, the charging head for the destruction equipment (renderer/tissue autoclave or alkaline hydrolysis) is usually inside of biocontainment and the operational side outside of biocontainment, to allow for easy servicing.

The method of decontamination/sterilization used must be validated to demonstrate the efficacy of the method. This can be challenging since ideally it is necessary to validate a process with the carcasses and the pathogens they might contain. Additionally, the air or effluent discharge must be acceptable to the local authorities (e.g., Publicly Owned Treatment Works) for release to the environment (e.g.,

temperature, biological or chemical oxygen demand) or further treated onsite to ensure agreed upon discharge parameters.

Facility Design and Caging

Facilities need to be designed to be able to contain the species of animal comfortably, according to animal welfare guidelines, and safely so that aerosols, effluent, solid waste and animal carcasses and tissues are not released accidentally from the facility to the environment, based upon the risk assessment. Increased concern over laboratory biosecurity has caused more attention to the facility's physical security features such as use of graded protection in the form of key and electronic door locks, visual monitoring and access control/monitoring.

Recent advances in animal welfare guidelines require greater space for animals, enrichment of their environment and better determination of endpoints for studies. Light can affect the physiology, morphology, and behavior of various animal species. Potential photo stressors include inappropriate photoperiod, photo intensity, and spectrum of light. Lighting should be diffused throughout an animal holding area and provide sufficient illumination for the well-being of the animals and to allow good housekeeping practices, thorough inspection of animals, and safe working conditions for staff.

Non-traditional laboratory animals such as reptiles, fish, amphibians, a variety of agricultural species (e.g., pigs, sheep, goats, chickens) and various wildlife species are now being used which require unique housing, enrichment and handling techniques. Due to the wide variety of animal species now being used, the housing requirement to provide animal comfort needs to be considered carefully in relation to biosafety. For example, certain species of birds require wooden branches for perching, cattle may require straw for bedding or mollusks may require mud, all of which conflict with good biosafety practices.

Whenever possible, house animals in primary containment to reduce the potential for room contamination. Advances in caging materials have allowed for a greater variety of species to be housed in species-appropriate containment caging.

Care should be taken when a species must be removed from its biocontainment caging to be manipulated as part of a study but cannot

be transferred directly into another ventilated enclosure. Greater protections may need to be taken by the animal care staff during these times and the status of the room itself may change from non-contaminated to potentially contaminated. In some cases stringent surface/room decontamination procedures will need to be put into place to address transient contamination issues.

Greater recognition of the risk of aerosol transmission of pathogenic agents has created greater need for individually ventilated caging, which is now provided by many manufacturers for many different species. When possible, low particulate dry bedding and cage pan liners should be used to minimize dust and aerosol creation. Animal isolators are now mobile, allowing biocontainment to be maintained while animals are either being moved between cages, biological safety cabinets, or between locations such as the animal room, procedure room, or laboratory.

Use of gaseous or vapor phase space decontamination (e.g., formaldehyde gas, vapor-phase hydrogen peroxide, chlorine dioxide gas) should be considered for cages, cage racks, and rooms as required.

When working with large animals infected with certain high consequence animal diseases, biocontainment to prevent escape of the pathogen into the environment becomes paramount. In these cases, facility design, and secondary and tertiary barriers become vital in containing the animal pathogen. The USDA Agricultural Research Service (ARS) 242.1, Facilities Design Standards and Appendix D - Agriculture Pathogen Biosafety of the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition provides important details in this area.

Biocontainment standards for large animals can be different than those for traditional laboratory animals because the environmental protections may be more important than the human health hazards. A guideline for consideration of biocontainment levels for animals infected with agricultural pathogens has been published. (Heckert and Kozlovac, 2007; OIE, 2008)

Vivarium Biosecurity of Animals Infected with Select Agents

In recent years a certain number of highly dangerous pathogens have been classified as "select agents" which require greater oversight and

accountability. Animal research and diagnostic activities involving these agents require a high degree of attention, resources and management in order to meet regulatory requirements.

Access to areas where animals exposed to select agents are housed must be carefully controlled and monitored. Only those people that meet federal and local security requirements are allowed to enter unescorted.

Inventory of animals exposed to select agents must be carefully monitored and all animals accounted for until appropriately decontaminated. Samples taken from animals infected with select agents must be inventoried and accounted for during all stages of removal, transport, testing and disposal. Any animals, tissues or samples taken for long-term storage must be tracked and recorded until their final destruction.

Program Management

Due to the complexity of many animal studies that can involve multiple hazards and their related oversight requirements, such as the use of transgenic animals, rDNA, infectious agents, and other potential harmful products, a thorough review process is necessary. Close coordination among the ACUC, IBC, and other oversight groups is required for a timely review process to occur. The IBC must contain the expertise necessary to assess the project, the risks involved and the biorisk mitigation measures employed.

Medical surveillance of veterinary, animal care, pathology and research staff is important to monitor for potential animal-induced allergies, injuries and accidental exposure to the research materials including potential biohazards. The National Research Council publishes recommendations on occupational health and safety in the care and use of research animals, including guidance on risks of specific zoonotic diseases and animal allergies.

The occupational risks should be risk based versus only exposure based and must involve an activity based risk assessment and a health based risk assessment. Vaccinations should be used as necessary based upon risk assessment to protect the animal care workers from serious diseases.

Great advances have occurred in engineering devices and safety equipment that can now be used to prevent staff from being exposed to the hazards associated with research. Based upon the risk assessment these devices should be used to prevent occupational exposures.

Anyone working with any species of animal must be well trained and experienced in handling the particular species of animal, as well as the biological, chemical and radiological hazards associated with the experiment. For example, someone used to working in a mouse colony would not be able to work with cows or horses, unless they received the necessary training and experience. Training and experience needs to be documented. For high hazard research, it is important that proficiency is demonstrated both for animal specific procedures and requirements for biosafety and general safety.

Advances in Integrated Pest Management (IPM) programs have allowed great improvements in pest control, which is especially important when doing infectious disease studies where insects can transmit or mechanically transport pathogens. The basic tenets of IPM include: a) monitoring for pests, b) identifying them, c) controlling them, and d) preventing their reoccurrence.

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3

Practicing Safe Science

Decontamination and Disposal

The goal of disinfection is not only the protection of personnel and the environment from exposure to biological agents, but also the prevention of contamination of experimental materials by the ubiquitous background of microorganisms. In the absence of adequate information, tests to determine the efficacy of candidate disinfectants should be conducted with the specific agent of interest. Also, the "need" for the level of decontamination being requested should be carefully examined; a surface decontamination with an appropriate liquid may well achieve the objectives with less risk and less work than a full space disinfection using a vapor or gas.

Physical and Chemical Disinfection

Physical and chemical means of disinfection fall into four main categories: Heat, Liquid Disinfectants, Vapors and Gases, and Radiation.

Heat The application of heat, either moist or dry, is recommended as the most effective method of sterilization. Steam at 121°C under 15 psi (1 bar) pressure in the autoclave is considered the most convenient method of rapidly achieving sterility. Inactivation of prions requires far higher pressure (132°C for 4.5 h in the presence of 1N NaOH). The most common reason for autoclaving not resulting in decontamination is a failure to provide access to the steam (e.g., sealing a bag of dry waste). Dry heat at 160° to 170°C for periods of 2 to 4 hours is suitable for destruction of viable agents on impermeable nonorganic material such as glass, but is not reliable in even thin layers of organic or inorganic material that can act as insulation. Incineration kills microorganisms and serves as an efficient means for disposal, but increasingly stringent

permitting requirements make the addition of an incinerator to a facility lacking one unlikely. Obtaining permission for an animal crematorium, using a two-chamber combustion system, may be feasible, but is not likely for general waste incineration.

Liquid Disinfectants. In general, the liquid disinfectants find their most practical use in surface treatment and, at sufficient concentration, as sterilants of liquid waste for final disposal in sanitary sewerage systems. There are many misconceptions concerning the use of liquid disinfectants. This is due largely to a characteristic capacity of such liquids to perform dramatically in the test tube and to fail miserably in a practical situation. Such failures often occur because proper consideration was not given to such factors as temperature, time of contact, pH, concentration, and the presence and state of dispersion, penetrability and reactivity of organic material at the site of application. Small variations in the above factors may make large differences in effectiveness of disinfection. For this reason, even when used under highly favorable conditions, complete reliance should not be placed on liquid disinfectants when the end result must be sterility.

There are many liquid disinfectants available under a wide variety of trade names. In general, these can be categorized as halogens, acids or alkalies, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols and amines. Unfortunately, the more active disinfectants often possess undesirable characteristics, such as corrosive properties. None is equally useful or effective under all conditions.

Vapors and Gases. A variety of vapors and gases possess germicidal properties. Historically, the most useful of these has been formaldehyde and ethylene oxide. Due to toxicity and carcinogenicity concerns, ethylene oxide has been relegated to a niche market for sterilization of medical instruments, which cannot be autoclaved. Over the past decade, progress has been made in commercializing the use of Vapor Phase Hydrogen Peroxide (VPHP) and Chlorine Dioxide (ClO_2) space sterilization systems. When these can be employed in closed systems and under controlled conditions of temperature and humidity, sterilization can be achieved. Vapor and gas disinfectants are primarily

useful in sterilizing: a) biological safety cabinets and associated effluent air-handling systems and air filters; b) bulky or stationary equipment that resist penetration by liquid surface disinfectants; c) instruments and optics that might be damaged by other sterilization methods; and d) rooms and buildings and associated air- handling systems.

Radiation. Ionizing radiation will destroy microorganisms. The germicidal action of x-rays has been known for over 100 years. Gamma radiation is used for the destruction of microorganisms in some food products and for the sterilization of certain medical products. Ionizing radiation is not a practical tool for laboratory use. However, ultraviolet radiation (UV) is a practical method for inactivating viruses, mycoplasma, bacteria and fungi. This nonionizing radiation is especially useful for the destruction of airborne microorganisms and, to a lesser extent, for the inactivation of microorganisms on exposed surfaces or for the treatment of products of unstable composition that cannot be treated by conventional methods. The usefulness of ultraviolet radiation as a sanitizer is limited by its low penetrating power, the need to maintain the cleanliness of the bulbs as dust and film reduces output, and the limited lifespan of the bulbs (2000 - 6000 hours). The NSF International currently recommends that individuals not install UV lamps in Class II biosafety cabinets, due to their limitations. UV can be a useful adjunct to chemical disinfection and can be the method of choice to inactivate potentially contaminating DNA for PCR studies.

Characteristics of Chemical Disinfectants in Common Use in Laboratory Operations

Those persons working with viable microorganisms will find it necessary to disinfect work areas and materials, equipment, and specialized instruments by chemical methods. Chemical disinfection is necessary because the use of pressurized steam, the most reliable method of sterilization, is not normally feasible for disinfecting large spaces, surfaces, and stationary equipment. Moreover, high temperatures and moisture often damage delicate instruments, particularly those having complex optical and electronic components.

Chemical disinfectants are available as powders, crystals, liquid

concentrates or compressed gases. Use concentrations must be determined and dilutions made as required. Chemical disinfectants that are gaseous at room temperature may be useful as space disinfectants. Others become gases at reasonably elevated temperatures and can act as either aqueous surface or gaseous space disinfectants.

Inactivation of microorganisms by chemical disinfectants may occur in one or more of the following ways: a) coagulation and denaturation of protein; b) lysis; c) inactivation of an essential enzyme by oxidation, binding, or destruction of enzyme substrate. The relative resistance to the action of chemical disinfectants can be substantially altered by such factors as concentration of active ingredient, duration of contact, pH, temperature, humidity, and presence of organic matter. Depending upon how these factors are manipulated, the degree of success achieved with chemical disinfectants may range from minimal inactivation of target microorganisms to sterility within the limits of sensitivity of the assay systems employed.

"The ideal disinfectant would be cheap, broadly effective, with excellent documentation of inactivation of a number of organisms, harmless to the environment, compatible with most materials, and with a simple means of measuring the active concentration of active agent. Unfortunately, no single chemical disinfectant or method exists."

– Paul J. Meechan, Ph.D., MPH

There are dozens of disinfectants available under a wide variety of trade names. In general, these disinfectants can be classified as acids or alkalies, halogens, heavy metal salts, quaternary ammonium compounds, phenolic compounds, aldehydes, ketones, alcohols, and amines. Unfortunately, the more active the disinfectant, the more likely it will possess undesirable characteristics. For example, peracetic acid is a fast-acting, universal germicide. However, in the concentrated state it is a hazardous compound that can readily decompose with explosive violence. When diluted for use, it has a short half-life, produces strong, pungent, irritating odors, and is extremely corrosive to metals. Nevertheless, it is such an outstanding germicide that it is commonly used in germ-free animal studies despite these undesirable characteristics.

The halogens are a most active group of disinfectants. Chlorine, iodine, bromine, and fluorine will rapidly kill bacterial spores, viruses, rickettsiae, and fungi. Free halogen is the effective agent. These disinfectants are effective over a wide range of temperatures. The halogens have several undesirable features. They combine readily with protein, so that an excess of the halogen must be used if proteins are present. Also, the halogens are somewhat unstable, especially at lower pH levels, so that fresh solutions must be regularly prepared. Finally, the halogens corrode metals. A number of manufacturers of disinfectants have treated the halogens to control some of these undesirable features. For example, sodium hypochlorite reacts with p-toluene sulfonamide to form Chloramine T, and iodine reacts with certain surface-active agents to form the popular iodophors. These "tamed" halogens are relatively stable, nontoxic, odorless, and less corrosive to metals. The buffering of these compounds, however, decreases their germicidal effectiveness. This trade-off is required when these compounds are used in metal pans or dunk tanks.

Ineffectiveness of a disinfectant is often due to the failure of the disinfectant to contact the microorganism rather than failure of the disinfectant to act. If one places an item in a liquid disinfectant, one can see that the item is covered with tiny bubbles. Of course, the area under the bubbles is dry, and microorganisms in these dry areas will not be affected by the disinfectant. Also, if there are spots of grease, rust or dirt on the object, or a biofilm, microorganisms under these protective coatings will not be contacted by the disinfectant. Scrubbing an item when immersed in a disinfectant is helpful, and a disinfectant should have, and most do have, incorporated surface-active agents.

Properties of Some Common Liquid Disinfectants

In general, liquid disinfectants are best suited for disinfection of hard surfaces or bulk liquids. They can be relatively inexpensive, can have broad-spectrum activity, but their compatibility with electronic or optical equipment has not been fully documented. Also, the contact time can run from minutes for enveloped viruses to hours for spores and it may require multiple re-applications to achieve sufficient contact time. Finally, considerable effort is required to perform a true inactivation

kinetics study for the specific agent(s) being inactivated in the specific matrix being cleaned (solid surface, bulk liquid, culture media, etc.). Each will likely require a different contact time and the label directions will only provide a starting point for the actual time and concentration required.

Alcohol. Ethyl or isopropyl alcohol in a concentration of 70 - 85% by weight is often used. Alcohols denature proteins and are effective disinfectants against lipid-containing viruses. They are also somewhat slow in their germicidal action and, if sprayed or wiped on a surface, evaporate quickly and may not provide adequate contact time.

Formaldehyde. Formaldehyde for use as a disinfectant is usually marketed at about 37% concentration of the gas in water solution referred to as formalin or as a solid polymerized compound called paraformaldehyde. Formaldehyde in a concentration of 5% active ingredient is an effective liquid disinfectant. Formaldehyde at 0.2 to 0.4% is often used to inactivate viruses in the preparation of vaccines. Formaldehyde loses considerable disinfectant activity at refrigeration temperatures. Formaldehyde exposure is strictly limited by OSHA and formaldehyde solutions should not be used as a surface disinfectant.

Phenol. Phenol itself is not often used as a disinfectant. The odor is somewhat unpleasant and a sticky, gummy residue remains on treated surfaces. This is especially true during steam sterilization. Although phenol itself may not be in widespread use, phenol homologs and phenolic compounds are basic to a number of popular disinfectants. The phenolic compounds are effective disinfectants against some viruses, rickettsiae, fungi and vegetative bacteria. The phenolics are not effective in ordinary usage against bacterial spores. The use of phenolic disinfectants has been reduced over the past two decades due to their residue issues, replaced by "quats" and chlorine-containing compounds.

Quaternary Ammonium Compounds or Quats. These cationic detergents are strongly surface-active and this detergency property makes them good surface cleaners. The Quats will attach to protein so that dilute solutions of Quats will lose effectiveness in the presence of

proteins. The Quats tend to clump microorganisms and are neutralized by anionic detergents, such as soap. The Quats are bacteriostatic, tuberculostatic, sporostatic, fungistatic and algistatic at low concentrations. They are bactericidal, fungicidal, algicidal and virucidal against lipophilic viruses at medium concentrations, but they are not tuberculocidal, sporicidal or virucidal against hydrophilic viruses even at high concentrations. The Quats have the advantages of being odorless, nonstaining, and noncorrosive to metals, stable, inexpensive and relatively nontoxic.

Micro-Chem Plus™ (NCL #0255) is a popular Quat introduced in 1994. It is a phosphate free, multi-purpose detergent disinfectant cleaner designed for controlling the hazard of cross contamination. Micro-Chem Plus provides exceptional broad-spectrum bactericidal, fungicidal and virucidal efficacy. It has become the agent of choice in many BSL-3 and BSL-4 research laboratories for decontaminating surfaces and Delta and Dover containment laboratory suits worn by BSL-4 scientists and technical staff. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) uses a 5% solution (190mL/gallon) of Micro-Chem Plus in all of its chemical showers. In a dilution of 1:64, it is also effective in the presence of organic soil (5% blood serum).

Chlorine. This halogen is a universal disinfectant active against all microorganisms including bacterial spores. Chlorine combines with protein and rapidly decreases in concentration in its presence. Free, available chlorine is an active element. It is a strong oxidizing agent, corrosive to metals. Chlorine solutions will gradually lose strength so that fresh solutions must be prepared frequently; the useful life of a 10% bleach solution is 24 hours, although "stabilized" solutions are commercially available. Sodium hypochlorite is usually used as a base for chlorine disinfectants. An excellent disinfectant can be prepared from household or laundry bleach. These bleaches usually contain 5.25% available chlorine or 52,500 ppm. If one dilutes them 1 to 100, the solution will contain 525 ppm of available chlorine; if a nonionic detergent is added in a concentration of about 0.7 percent, a very good disinfectant is created.

The halogenated byproducts of chlorine and organic materials have recently raised concerns regarding potential toxicity or carcinogenicity.

Prior to the widespread use of chlorine compounds at a facility, there needs to be an evaluation of the amount of chloroform and chloramine, which could be generated and discussed with the local waste water treatment authority. Also, autoclaving chlorinated solutions will cause the halogen to attack the stainless steel, potentially shortening the lifespan of the pressure vessel.

Iodine. The characteristics of chlorine and iodine are similar. One of the most popular groups of disinfectants used in the laboratory is the iodophors, and Wescodyne is perhaps the most widely used. The range of dilution of Wescodyne recommended by the manufacturer is 1 oz. in 5 gal. of water giving 25 ppm of available iodine to 3 oz. in 5 gal., giving 75 ppm. At 75 ppm, the concentration of free iodine is 0.0075 percent. This small amount can be rapidly taken up by extraneous protein present. Clean surfaces or clear water can be effectively treated by 75 ppm available iodine, but difficulties may be experienced if any appreciable amount of protein is present. For washing the hands or for use as a sporicide, it is recommended that Wescodyne be diluted 1 to 10 or 10% in 50% ethyl alcohol, which will give 1,600 ppm of available iodine. At this concentration, relatively rapid inactivation of any and all microorganisms will occur.

Peroxy Acids and Peracetic Acid. As a class, peroxy acids and peracetic acid-based disinfectants have become popular, especially in animal facilities. They are effective against most organisms, including spores. However, the contact time can be extensive for spores, ranging from 30 min to 5.5 h, which mandates numerous re-applications to ensure inactivation of spores. The peracetic acid disinfectants have a strong odor, which may be objectionable to some staff. The peroxy acid-based disinfectants can also leave a visible residue and must be rinsed off when a clean surface is required.

Vapors and Gases for Space Decontamination

Chemical disinfectants that have been used as space decontaminants include formaldehyde, ethylene oxide, peracetic acid, VPHP, ClO_2, and glutaraldehyde. When these can be used in closed systems and under

controlled conditions of temperature and humidity, excellent disinfection can be obtained. Ethylene oxide adsorbed by materials such as rubber must be removed by aeration and considered carcinogenic. It is also convenient to use, versatile, and noncorrosive, which explains its ongoing use for medical equipment. Peracetic acid is corrosive for metals and rubber and is more frequently used as a liquid disinfectant. All space disinfectants need to be validated for kill through the use of biological indicators, such as *G. stearothermophilis* for hydrogen peroxide or *B. atrophaeus* for formaldehyde.

Formaldehyde. Formaldehyde has been, in general, the chemical of choice for space disinfection for the past half century, and for many institutions, will remain so for the foreseeable future. Biosafety cabinets, incubators, refrigerators, laboratory rooms, buildings, or other enclosed spaces can be disinfected with formaldehyde. The formaldehyde can be generated from aqueous solutions (formalin) containing 37 - 40% formaldehyde by heating or by vaporizing the solution. Formaldehyde gas, also, can be generated by heating paraformaldehyde, which is a solid polymer that contains 91 - 99% formaldehyde. If aqueous formaldehyde is used, the application rate should be one milliliter for each cubic foot of space to be treated. The facility/equipment must be sealed to prevent leaking of the gas. To assure thorough mixing, the use of air-circulating fans may be required. Areas being treated should have a temperature of at least 10°F (21°C) and a relative humidity of above 70%. Spaces being treated should not be wet, have condensate on the walls, or have pools of water on the floor, since formaldehyde is quite soluble in water and will be rapidly taken up. Also, as the water evaporates, polymerization will take place on the surfaces and these polymers are difficult to remove. Formaldehyde is a powerful reducing agent and is noncorrosive to metals. It can normally be assumed that any equipment or apparatus that will not be damaged by the humidity necessary for decontamination will not be damaged by the formaldehyde. Although formaldehyde is a true gas and will sterilize all exposed surfaces, it has limited penetrating abilities, and materials that are tightly covered may not be sterilized.

Generally, the generation of formaldehyde gas from prills, powdered, or flake paraformaldehyde by heating is the preferred

method. Paraformaldehyde will depolymerize and convert to the gaseous state when heated to a temperature above 150°C (302°F). There are various practical methods for heating the paraformaldehyde to above 150°C, but a commercially available electric frying pan equipped with a thermostat is one of the simplest. The electric cord of the frying pan should be equipped with a one-hour timer so that the pan can be placed in the space to be treated and, after the sublimation of the formaldehyde gas, the power to the frying pan will be turned off automatically. An average frying pan can hold one kilogram of flake formaldehyde. The depolymerization rate of paraformaldehyde is about 20 g per minute when the thermostat is set at 232°C (450°F). A concentration of 0.3 g of paraformaldehyde for each cubic foot of space to be treated is employed. Temperature of the space must be above 20°C and relative humidity 70% or higher. Exposure times need to be at least two hours and, if possible, the exposure should be for eight hours or overnight. Formaldehyde generated from paraformaldehyde has better penetration, and fewer problems with condensation and subsequent need for prolonged aeration, than with formaldehyde generated from formalin. If walls and surfaces were not wet with condensation during the formaldehyde treatment process, then neutralization with heated ammonium carbonate or bicarbonate, followed by aeration and removal of the formaldehyde should proceed rapidly. Neutralization requires the heating of an equivalent amount of either ammonium compound to generate enough ammonia to react with all of the formaldehyde in the room (plus 10% for a safety margin). The ratio generally used is 1.2g $(NH_4)_2CO_3$/ft³ or 1.6g NH_4HCO_3/ft³ and, like paraformaldehyde, is heated in a fry pan at 232°C (450°F). A small room with nonporous surfaces and no materials or equipment in the room can be cleared of all detectable formaldehyde in less than an hour of aeration. However, an entire building containing a variety of surfaces and equipment may take many hours or even a day or more of aeration to remove the formaldehyde. After aeration and determination that the residual formaldehyde concentration is below 0.05 ppm, the area may be entered wearing appropriate PPE, all surfaces wiped down with wet cloths to remove any residual film, and then the area may be re-occupied.

Formaldehyde is a toxic substance having a threshold limit value (TLV) of 2 ppm. Considerable caution must be exercised in handling,

storing and using formaldehyde. Repeated exposure to formaldehyde is known to produce a hypersensitive condition in certain individuals. Self- contained breathing apparatus, or full face respirators fitted with formaldehyde-specific cartridges must be available and used whenever exposure to formaldehyde is possible. Most individuals can readily detect formaldehyde in a concentration of 1 ppm, which is sufficiently close to the OHSA limit that nose detection should not be used as a method of determining a "safe" level. Formaldehyde measuring devices (e.g., Dräger tubes or a formaldehyde meter) should be used. Any person using formaldehyde to decontaminate space must be enrolled in appropriate medical surveillance and have written protection plans and monitoring (as per 29 CFR 1910.1048 Formaldehyde). Formaldehyde may combine with hydrochloric acid to form bis (chloromethyl) ether, a compound that is carcinogenic. When formaldehyde is to be used as a space disinfectant, the area to be treated should be surveyed to insure that there are no open containers of any acidic solution containing chloride ion. It should be mentioned that formaldehyde in the concentrations used for space disinfection has no effect on cockroaches or possibly on other insects or arachnids as well.

Formaldehyde is explosive at concentrations between 7.0 and 73.0% by volume in air. This concentration, however, should not be reached when standard procedures are used, but demands care when calculating the amount of paraformaldehyde to use; an error in calculation of an order of magnitude (factor of 10) would result in using an amount close to the lower explosive limit.

In the US, the Environmental Protection Agency regulates disinfectants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). There is, as of this writing, no formaldehyde solutions or solid paraformaldehyde compounds registered for space decontamination under FIFRA.

Vapor Phase Hydrogen Peroxide. Although the use of vapor phase hydrogen peroxide (VPHP) has increased due to the shorter exposure time and easier cleanup (H_2O_2 breaks down to H_2O and O_2), the cost of specialized machinery (up to $60,000 per machine, plus ongoing costs for proprietary reagents) have slowed the spread in institutions with limited capital funds.

Condensation remains a potential issue, even within interior rooms (condensation on exterior walls, especially exterior windows, is a problem with any vapor-based system). Reduction of relative humidity to 30 - 40% reduces, but does not eliminate this issue. Since VPHP is a vapor and not a gas, it does not distribute well in a closed area and requires either a distribution system or fans situated through the area being decontaminated in order to assure uniform distribution of the compound. The effective concentration required for disinfection is approximately 2.4mg/l for 1h. With the PEL at 1.4 ug/l (1.0 ppm) and neither OSHA or NIOSH reporting an odor threshhold, the sealing and monitoring of the facility is extremely important, as is the wearing of appropriate PPE. Despite these issues, this system is becoming the method of choice outside the US, especially in the EU and Canada.

Chlorine Dioxide. Gaseous chlorine dioxide (ClO_2) is competing with hydrogen peroxide as a replacement for formaldehyde. Like VPHP, ClO_2 has the advantage of a shorter cycle time, using a usual concentration of 10-30 mg/l for 2h. Like paraformaldehyde, an increased relative humidity (60 - 75%) is required for optimal disinfection. ClO_2 unit costs are intermediate to those required for paraformaldehyde and VPHP; a unit can cost approximately $10,000, plus the ongoing costs for proprietary reagents. As with H_2O_2, the unit both generates the disinfectant and scrubs it from the air. Similar to formaldehyde, if a person can detect ClO_2 they have reached the PEL, as the PEL for ClO_2 is 0.3 ug/l (0.1 ppm) and the odor threshold is 0.1 ppm. As with the other space disinfectants, it requires sealing and monitoring of the facility, as well as proper PPE. As a true gas, however, the penetrating power is higher than VPHP and is similar to formaldehyde.

Residual Action of Disinfectants

As noted in the preceding discussion of disinfectant properties, some of the chemical disinfectants have residual properties that may be considered a desirable feature in terms of aiding in the control of back ground contamination. One is cautioned, however, to consider residual properties carefully. Ethylene oxide used to sterilize rubber products may be adsorbed by the rubber and desorbed slowly. Therefore, if the

rubber products (shoes, gloves, respirators} are not thoroughly aerated (e.g., at least 24 hours), the ethylene oxide leading the rubber material that is in contact with the skin may cause severe skin irritation. As noted above, areas of condensation can result in pools of formalin or patches of paraformaldehyde after use of formaldehyde space disinfection, or H_2O_2 after VPHP. Cell cultures, as well as viruses of interest, may be inhibited or inactivated by disinfectants persisting after routine cleaning procedures. Therefore, reusable items that are routinely held in a liquid disinfectant prior to autoclaving and cleaning should receive particular attention in rinse cycles. Similarly, during general area sterilization with gases or vapors, it may be necessary to protect new and used clean items such as glassware, by removing them from the area or by enclosing them in gastight bags or by insuring adequate aeration following sterilization.

Laboratory Spills

A problem that may occur in the laboratory is an overt biological spill. A spill that occurs in the open laboratory may create a serious problem. Every effort should be taken to avoid such occurrences. A spill poses less of a problem if it occurs in a biological safety cabinet provided splattering to the outside of the cabinet does not occur. Direct application of concentrated liquid disinfectant and a thorough wipe down of the internal surfaces of such cabinetry will usually be effective for decontaminating the work zone, but gaseous sterilants will be required to disinfect the interior sections of the cabinet. Each researcher must realize that in the event of an overt accident, research materials such as tissue cultures, media, and animals within such cabinets may well be lost to the experiment.

Spill in a Biological Safety Cabinet. A spill that is confined to the interior of the biological safety cabinet should present little or no hazard to personnel in the area. However, chemical disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet. For a pool of contaminated material, absorb the material with dry paper towels. Carefully pour concentrated disinfectant, starting at the outside of the spill. For dispersed or splattered materials, wipe walls, work

surfaces, and equipment with a disinfectant. A disinfectant with a detergent has the advantage of detergent activity, which will help clean the surfaces by removing both dirt and microorganisms. When in doubt regarding the efficacy of the standard disinfectant at the facility, a 1:10 dilution of household bleach is a suitable general disinfectant. The operator should wear PPE, including gloves, during this procedure.

If the splash/splatter is generalized, use sufficient disinfectant solution to ensure that the drain pans and catch basins below the work surface contain the disinfectant (make sure the drain from the catch basin is closed first!). Lift the front exhaust grill and tray and wipe all surfaces. Wipe the catch basin and carefully drain any excess disinfectant into a container, taking care not to splash the disinfectant into the collection bucket. The gloves, wiping cloth and sponges should be discarded into an autoclave pan and autoclaved. This procedure will not disinfect the filters, blower, air ducts or other interior parts of the cabinet.

If the entire interior of the cabinet is to be sterilized, this can be accomplished by the formaldehyde gas method described above. Place the paraformaldehyde in the frying pan and place the pan in the cabinet with the electric line run to the outside of the cabinet. Raise the humidity within the cabinet to about 70%. Vaporization of water in the frying pan is a convenient technique. Set the thermostat of the frying pan containing the paraformaldehyde at 232°C (450°F). Seal the cabinet opening with sheet plastic and tape. If the cabinet exhaust air is discharged into the room, attach the flex hose to the cabinet exhaust port and extend the hose back into the cabinet opening and seal the gap with tape. If the cabinet is exhausted directly into the building system, close the exhaust damper. Plug in the frying pan to depolymerize the paraformaldehyde. After one-half volume of paraformaldehyde has been depolymerized, turn on the cabinet fan for about three seconds to allow the formaldehyde gas to reach all areas. After depolymerization is complete, again turn on the cabinet fan for three seconds. Allow the cabinet to stand for a minimum of one hour. After the one-hour exposure, neutralize the formaldehyde with ammonium carbonate or bicarbonate. Carefully remove the flex hose and attach it to the exhaust damper, slit the plastic covering the opening and turn on the cabinet fan. Ventilate the cabinet for several hours to remove all traces of formaldehyde.

Spill in the Open Laboratory. A plan for containing spills must be developed in advance. If potentially hazardous biological material is spilled in the laboratory, the first essential is to minimize inhaling any airborne material by holding the breath and leaving the laboratory. Warn others in the area and go directly to a wash or change room area. If clothing is known or suspected to be contaminated, remove the clothing with care, folding the contaminated area inward. Discard the clothing into a bag or place the clothing directly in an autoclave. Wash all potentially contaminated areas as well as the arms, face and hands. Shower if facilities are available. Reentry into the laboratory should be delayed for a period of 30 minutes to allow reduction of the aerosol generated by the spill. Advance preparation for management of a spill is essential. A "spill kit," including leak-proof containers, forceps, paper towels, sponges, disinfectant, respirators, rubber gloves and other PPE as dictated by your risk assessment, should be readily available.

Protective clothing should be worn when entering the laboratory to clean the spill area. Gloves compatible with the disinfectant to be used, autoclavable or disposable footwear, an outer garment (preferably a disposable jumpsuit) and, if appropriate and fit tested, a respirator should be worn. Take the "spill kit" into the laboratory room, place a discard container near the spill, and transfer large fragments of material into it; replace the cover. If broken glass or other sharps are present, do not pick them up by hand; use a pair of tongs or a brush and piece of cardboard. Then cover all visible spilled material using paper towels or other absorbent, as needed for the size of the spill. Be generous in estimating the size of the spill to take into account splash. Using a hypochlorite containing 5000 ppm available chlorine (1:10 dilution of household bleach) or other appropriate disinfectant, carefully pour the disinfectant around and into the absorbed spill. (These concentrations of disinfectants are higher than those normally employed in the laboratory because the volume of spill may significantly reduce the concentration of active ingredient in the disinfectant.) Avoid splashing. Allow 15 minutes contact time. Use additional paper or cloth towels to wipe up the disinfectant and spill, working toward the center of the spill. Discard towels into a discard container as they are used. Wipe the outside of the discard containers, especially the bottom, with a towel soaked in a disinfectant. Place the discard container and other materials in an

autoclave and sterilize. Remove shoes, outer clothing, respirator and gloves and sterilize by autoclaving or exposure to ethylene oxide. Wash hands, arms and face or, if available, shower. If gaseous disinfection of the laboratory room is to be carried out, follow the procedures as outlined above in Vapors and Gases for Space Decontamination.

Radioactive Biohazard Spill Outside a Biological Safety Cabinet. In the event that a biohazardous spill also involves radiation hazard, the cleanup procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard. Laboratories handling radioactive substances will have the services of the designated radiation area supervisor to aid in the cleanup. Before cleanup procedures begin, a radiation protection officer should survey the spill for external radiation hazard to determine the degree of risk. In most cases, the spill will involve C or ^{3}H, which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The radiation protection officer must make this determination before the cleanup operation is begun.

If the radiation protection officer approves, the biohazard handling procedure may begin: Using an autoclavable dust pan and squeegee, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a deep autoclave pan. Sufficient disinfectant solution to immerse the contents should be added to the waste container. The cover is to be replaced on the pan, the pan should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive waste. Radioactive and biohazard warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided. A final radioactive survey should be made of the spill area cleanup tools, and shoes and clothing of individuals who had been in the area by taking swipes and counting in an appropriate counter.

Disposal

Decontamination and disposal in infectious disease laboratories are closely interrelated acts in which disinfection constitutes the first phase of disposal. All materials and equipment used in research will

ultimately be disposed of; however, in the sense of daily use, only a portion of these will require actual removal from the laboratory complex or onsite destruction. The remainder will be recycled, either for use within the same laboratory or in other laboratories, or for the value of their material (e.g., recycling plastic pipet tip boxes for the polypropylene). Examples of the former are: reusable laboratory glassware, instruments used in necropsy of infected animals, and laboratory clothing. Disposal should therefore be interpreted in the broadest sense of the word, rather than in the restrictive sense of dealing solely with a destructive process.

The principal questions to be answered prior to disposal of any objects or materials from laboratories dealing with potentially infectious microorganisms or animal tissue are:

a. Have the objects or materials been effectively disinfected or sterilized by an approved procedure?
b. If not, have the objects or materials been packaged in an approved manner for immediate onsite incineration or transfer to another laboratory?
c. Does disposal of the disinfected or sterilized objects or materials involve any additional potential hazards, biological or otherwise, to those carrying out the immediate disposal procedures or those who might come into contact with the objects or materials outside the laboratory complex?

Laboratory materials requiring disposal will normally occur as liquid, solids and animal room wastes. The volume of these can become a major problem when there is the requirement that all wastes be disinfected prior to disposal. It is most evident that a significant portion of this problem can be eliminated if the kinds of materials initially entering the laboratory are reduced. In any case, and wherever possible, materials not essential to the research should be retained in the non-research areas for disposal by conventional methods. Examples are the packaging materials in which goods are delivered, disposable carton cages for transport of animals, and large carboys or tanks of fluids that can be left outside and drawn from as required. Reduction of this bulk will free autoclaves for more rapid and efficient handling of materials

known to be contaminated.

Inevitably, disposal of materials raises the question, "How can we be sure that the materials have been treated adequately to assure that their disposal does not constitute a hazard?" In the small laboratory, the problem is often solved by having each investigator disinfect all contaminated materials not of immediate use at the end of each day and place them in suitable containers for routine disposal. In larger laboratories, where the mass of materials for disposal becomes much greater and sterilization bottlenecks occur, materials handling and disposal will likely be the chore of personnel not engaged in the actual research. In either situation, a positive method should be established for designating the state of materials to be disposed. This may consist of a tagging system stating that the materials are either sterile or contaminated.

Disposal of materials from the laboratory and animal holding areas will be required for research projects ranging in size from an individual researcher to those involving large numbers of researchers in many disciplines. Procedures and facilities to accomplish this will range from the simplest to the most elaborate. The primary consideration in any of these is to dispel the notion that laboratory wastes can be disposed of in the same manner, and with as little thought as household wastes. Selection and enforcement of safe procedures for disposal of laboratory materials are of no less importance than the consideration given to any other methodology for the accomplishment of research objectives.

Materials of dissimilar nature will be common in laboratories. Examples are combinations of common flammable solvents, chemical carcinogens, radioactive isotopes, and concentrated viruses or nucleic acids. These may require input from a number of disciplines in arriving at the most practical approach for their decontamination and disposal.

Selecting Chemical Disinfectants in Research

The ideal disinfectant would be cheap, broadly effective, with excellent documentation of inactivation of a number of organisms, harmless to the environment, compatible with most materials, and with a simple means of measuring the active concentration of active agent. Unfortunately, no single chemical disinfectant or method will be effective or practical for

all situations in which decontamination is required. Selection of chemical disinfectants and procedures must be preceded by practical consideration of the purposes for the decontamination and the interacting factors that will ultimately determine how that purpose is to be achieved. Selection of any given procedure will be influenced by the information derived from answers to the following questions:

a. What is the target organism(s)?
b. What disinfectants, in what form, are known to, or can be expected to, inactivate the target organism(s)?
c. What degree of inactivation is required?
d. In what menstruum is the organism suspended (i.e., simple or complex, on solid or porous surfaces, and/or airborne)?
e. What is the highest concentration of cells/viruses/etc. anticipated to be encountered?
f. Can the disinfectant, either as a liquid, a vapor, or gas, be expected to contact the organisms, and can effective duration of contact be maintained?
g. What restrictions apply with respect to compatibility of materials?
h. What is the stability of the disinfectant in use concentrations, and does the anticipated use situation require immediate availability of the disinfectant or will sufficient time be available for preparation of the working concentration shortly before its anticipated use?

The primary target of decontamination in the laboratory is the organism(s) under investigation. Laboratory preparations or cultures usually have titers in excess of those normally observed in nature. Inactivation of these materials presents other problems, since agar, proteinaceous nutrients, and cellular materials can effectively retard or chemically bind the active moieties of chemical disinfectants. Such interferences with the desired action of disinfectants may require higher concentrations and longer contact times than those shown to be effective in the test tube. Similarly, a major portion of the contact time required to achieve a given level of agent inactivation may be expended in inactivating a relatively small number of the more resistant members of

the population. The current state of the art provides little information on which to predict the probable virulence of these more resistant cells. These problems are, however, common to all potentially pathogenic agents for their use.

Organisms exhibit a range of resistance to chemical disinfectants. In terms of practical decontamination, most vegetative bacteria, fungi, and lipid containing viruses are relatively susceptible to chemical disinfection. The nonlipid containing viruses and bacteria with a waxy coating, such as mycobacteria, occupy a midrange of resistance. Spore forms are more resistant and prions are most resistant to disinfection.

A disinfectant selected on the basis of its effectiveness against organisms on any range of the resistance scale will be effective against organisms lower on the scale. Therefore, if disinfectants that effectively control spore forms are selected for routine laboratory decontamination, it can be assumed that any other organisms generated by laboratory operations, even in higher concentrations, would also be inactivated.

Pertinent characteristics and potential applications for several categories of chemical disinfectants most likely to be used in the biological laboratory are summarized in the following table. Practical concentrations and contact times that may differ markedly from the recommendations of manufacturers of proprietary products are suggested. It has been assumed that microorganisms will be afforded a high degree of potential protection by organic menstruums. It has not been assumed that a sterile state will result from application of the indicated concentrations and contact times. It should be emphasized that these data are only indicative of efficacy under artificial test conditions. Individual investigators should conclusively determine the efficacy of any of the disinfectants. It is readily evident that each of the disinfectants has a range of advantages and disadvantages as well as a range of potential for inactivation of a diverse microflora. Equally evident is the need for compromise as an alternative to maintaining a veritable "drug store" of disinfectants.

Caution Required When Applying Disinfectant Methods

Heat Sterilization. The hazards of handling hot solids and liquids are reasonably familiar. Laboratory personnel should be cautioned that steam under pressure could be a source of scalding jets if the equipment for its application is mishandled. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer too promptly after treatment. This can cause a sudden and violent boiling of the contents from containers that can splash scalding liquids onto personnel handling the containers. Appropriate PPE (including splash goggles, apron and heat resistant gloves) should be worn, as dictated by your site's risk assessment program.

Liquid Disinfectants. Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel making up use-concentrations from stock solutions must be properly informed as to the potential hazards and trained in the safe procedures to follow. The concentrated quaternary and phenolic disinfectants are particularly harmful to the eyes and skin. Even a small droplet splashed in the eyes may cause blindness. Protective face shields and goggles should be used for eye protection, and long-sleeved garments and chemically resistant gloves, aprons, and boots should be worn to protect from corrosive and depigmentation effects to the skin. One of the initial sources for hazard information on any given product will be the label on its container and the Safety Data Sheet. They must be available to any person working with these chemicals.

Vapors and Gases. Avoid inhalation of vapors of formaldehyde and ethylene oxide. Stock containers of these products should be capable of confining these vapors and should be kept in properly ventilated chemical storage areas in the event of inadvertent leakage. In preparing use-dilutions and when applying them, personnel should control the operations to prevent exposure of others and wear respiratory protection as necessary. Both ethylene oxide and formaldehyde are considered carcinogens; toxic and hypersensitivity effects are also well established for formaldehyde.

Radiation. The uses of UV irradiation carry the danger of burns to the cornea of the eyes and the skin of persons exposed for even a short time. Proper shielding must be maintained where irradiation treatment is used when personnel and laboratory animals are present. Guard against reflecting surfaces (e.g., polished stainless steel) occurring in line with the light source. In areas irradiated without shielding on special occasions or during off-duty hours, post the area with warning signs to prevent unscheduled entry of personnel. If a laboratory must use room UV, consider interlocking the switch with the door in order to turn off the lights if the door is accidentally opened. Users of biosafety cabinets with UV sources should minimize the use of UV. An hour of UV will inactivate most known agents (except prions); excess use damages plastics without additional positive results.

4

Practicing Safe Science

Biological Safety Cabinets

This discussion assumes a basic understanding of Class II bio cabinetry. There are many good reference sources for cabinet descriptions and intended purposes of each. One example is Appendix A - Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets of the CDC/NIH publication *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition. Class II biological safety cabinet (BSC) classification designations were modified slightly in 2002, but for the most part, no new Class II cabinet types have been added since the Type B2 was added in 1979. This section will focus on the lessons learned and the key changes to BSC practices since the early 1970s.

Class II BSCs were initially designed for use with particulate hazards only. The first designs were what we now call the Class II Type A2 cabinet (vented to the laboratory). They were used for working with biohazards while work with volatile hazards was strictly relegated to the laboratory fume hood. The BSCs then and now rely on HEPA filters to filter particulate hazards and provide unidirectional airflow. HEPA filters are limited to filtering particulate hazards while gases and vapors pass freely through. Eventually the need to combine volatile materials with bio hazardous work required alternative designs to the Type A cabinet, with external venting of exhaust air.

Externally venting Type A2 cabinets and using Type B1 and B2 cabinets adds significantly to the difficulty and cost of laboratory installations. Simplicity of design and operation should be embraced whenever possible. To this end, Class II Type A2 cabinets recirculated back into the laboratory offer the simplest and most cost effective installation and should be considered a primary choice any time potentially volatile materials will not be used. When external venting is required, the Class II Type A2 cabinet is the simplest design from an

operational perspective. The A2 cabinet is connected to the building exhaust system with a canopy connection.

In the early days, Type A cabinets were connected to the building exhaust system with a direct or solid connection. A direct connected Type A cabinet requires precise balance between the building exhaust blower and the cabinet blower. Any change to the building air balance, either supply or exhaust is going to impact the BSC performance. Opening and closing laboratory doors, changing airflow to other ventilation devices, worn belts, all directly impact the product, personnel, and environmental safety afforded by the cabinet. The canopy connection was developed to combat these problems. Originally called a "thimble" connection, the canopy connection introduces a gap between the cabinet exhaust HEPA filter and the transition to the duct. This gap allows in inflow of air in addition to the cabinet exhaust air, thus insulating the BSC performance from variations in the room environment and building ventilation system. It wasn't until 1992, however, that the biosafety community embraced the canopy design concept.

Prior to 1992, methodologies did not exist to effectively measure the inflow velocity of a canopy connected BSC. The development of the "Direct Inflow Measurement" (DIM) procedures allows for measurement of intake airflow volume directly at the face opening. Before that, the only means of determining intake velocity was to measure exhaust velocity and convert that to intake. Exhaust velocity cannot be accurately measured on a canopy-connected cabinet because of the gap at the cabinet discharge. Therefore, the canopy connection was generally relegated to secondary status. With the acceptance of DIM methodologies, the canopy connection gradually became more widely used. In 2002, the standard for Class II Biosafety Cabinets – NSF/ANSI 49, Biosafety Cabinetry: Design, Construction, Performance and Field Certification – stated that all Type A cabinets vented to the outside *should* be canopy connected. In 2010, NSF/ANSI 49 changed the word *should* to *shall*. It is now mandatory for compliance with NSF/ANSI 49 that externally vented Type A cabinets employ canopy connections. Direct connected Type A cabinets are no longer considered acceptable under NSF/ANSI 49 and whenever they are found in the field, the direct

connection should be converted to a canopy connection in order to maintain compliance with NSF/ANSI 49 requirements.

In the vast majority of cases properly vented Type A2 cabinets are adequate for handling the concentrations of volatile hazardous substances associated with biosafety applications. Certain specialized applications, such as anesthetizing animals, utilize large amounts of highly volatile hazardous substances, and represent legitimate reasons to consider the use of the much more complicated B2 cabinet design.

Unfortunately, a systematic approach to determining if a Type B2 cabinet is truly justified is not generally followed. All too often, the user assumes if a Type A2 cabinet is okay, a B1 cabinet must be better, and a B2 cabinet is surely the best for all applications. This is simply not true. The volatility of the substance along with the concentration should be weighed against the internal cabinet dilution rate. While the B2 cabinet will have the highest dilution rate of all class II cabinets, the dilution rates for Type A2 cabinets are rather high as well. Based on a 270 cubic foot per minute (CFM) exhaust volume for a typical 4' class II Type A2 BSC and a work chamber volume of 21 FT^3, a chamber turnover of 771 Air Changes Per Hour (ACPH) is created. A typical B2 cabinet exhausts 800 CFM with the same chamber volume resulting in a chamber turnover of 2,286 ACPH. Most BSC manufacturers are not willing to take responsibility for cabinet selection especially when volatile hazardous substances are involved. An industrial hygienist should perform a risk assessment based on anticipated volatility generation and work area air exchange rates. The B2 cabinet turns over approximately three times the amount of air that an A2 cabinet does but the A2 cabinet's high turnover

"Having the biosafety professional or someone from the occupational safety and health group sit down with the principal investigator or other folks in the lab who are looking to procure a new BSC is important. There should be a discussion to make sure the appropriate kind of cabinet is selected and that it can actually work in the space where the lab wants to place it. A centralized approval process involving the biosafety professional would have value."

– Jason Barr, CDR USPHS, MS, CIH

rate will satisfy most applications. In addition to the complexity penalty for Type B2 cabinets, the cost of energy and stress on building infrastructure must be considered.

When a proper risk assessment has been done and it is determined that a B2 or B1 cabinet is needed, the HVAC designer and air balancer must be made aware of the proper airflow set points to use in their planning. Up until very recently planners were provided the nominal set point values used to certify cabinet airflow for establishing exhaust airflow volume and duct static pressure. Airflow certification practices have evolved greatly since the 70s. Originally, all airflow testing was done with a thermal anemometer or a pitot tube and manometer. Exhaust airflow velocity was measured to determine exhaust volume, which was converted to intake velocity. These methods are considered accurate but they are not always repeatable. To further complicate issues, a straight section of exhaust duct is needed to obtain accurate exhaust readings but is very rarely found in real life laboratory situations.

The DIM was embraced as the primary method of determining face velocity because it is extremely repeatable and it takes practically no skill to perform. A capture hood is sealed to the BSC access opening and a direct intake airflow volume reading is taken. It is simple and repeatable. A factory technician at the BSC manufacture can take a measurement and expect that a field certification technician will be able to repeat his results. This is in contrast to performing a duct traverse. When two different technicians perform duct traverses with either a thermal anemometer or a pitot tube, the skill of the technicians will dictate how close their results will be. The test that requires no skill is a more reliable test than one that requires a high skill level; therefore, NSF incorporated the DIM as the primary method.

Engineers and air balancers still need to design their exhaust systems using traditional methodologies. This requires two values; exhaust airflow volume and duct static pressure. Since 2010, NSF publishes a "Concurrent Balance Value (CBV)" along with the certification set points for airflow testing. The engineers should be looking for the CBV when designing systems and the balancers should set the exhaust volume based on these values. The CBV is used only for Type B1 and B2 (direct connected) cabinets. The CBV listed is the nominal set point value,

which would be at the midrange of the acceptable exhaust airflow volume range.

In addition to the exhaust volume, a duct static pressure is listed. This is the anticipated *loaded* duct static pressure measured immediately downstream of the exhaust HEPA filter prior to the exhaust damper. It is important to engineers and to air balancers but used differently by both. The loaded duct static pressure is obtained by taking the static pressure reading with clean HEPA filters and adding a loading value to represent what the duct pressure will be when the HEPA filters become dirty. A loading value of 0.3" for a Type B1 and 0.7" for a Type B2 cabinet is added to the clean filter reading.

The intent of the CBV is to eliminate the confusion created when air balancers use a different method than the certifier while testing the same equipment. Historically, confrontations would occur because both were taking accurate readings but they did not agree. Now, the different methods are matched with different but related acceptance criteria. An engineer will use the CBV with full or loaded static pressure to design the system with adequate capacity to accommodate filter loading. The air balancer should expect to see the listed static pressure minus the loading value when performing initial airflow setup to the CBV. The certifier will then test to the nominal set point using a DIM or approved alternative method. If the system is designed and balanced to the certification values instead of the CBV, the airflow will be set too low to be certified.

Type B cabinets rely on an alarm and an interlock between the exhaust monitor and the supply fan to prevent dangerous consequences of the exhaust system operating at less than the acceptable range. In 2002, specific parameters and tests to prove compliance were added to NSF/ANSI 49. When the exhaust airflow volume decreases by 20% or more, an audible and visual alarm shall signal and the supply fan shall turn off. Site installation assessment tests have been added to the field testing section of Standard 49. These tests make ensuring performance of the alarms and interlocks obligatory components of the certification process.

Exhaust alarms are mandatory for all externally vented cabinets including the canopy connected A2. Interlocks are required on Type B cabinets but not Type A cabinets. For type A, one would usually want the fan to continue to run, thus maintaining containment of both gasses and particles at the front access opening closer to the user's breathing zone. Exhaust air, however, may then be returned to the laboratory. While HEPA filtered, volatiles still would be released. The requirement for an alarm alerts the user to this condition, allowing for appropriate response to stop work if volatiles are in use. The BSC manufacturer must verify the performance of the canopy design. They must prove that when the exhaust fan fails, the BSC face velocity does not decrease to an unsafe level. The canopy connection provides the safety that the interlocks provide to the B2 cabinets. This assumes only canopies manufactured by the BSC manufacturer are used. Site-made canopy connections that have not been validated should be avoided.

NSF/ANSI 49 continues to evolve. An online tool for tracking these changes is available at www.cetainternational.org. Additionally, CETA developed an applications guide (CAG-007-2010) to assist facilities engineers when designing Class II Type B exhaust systems.

5

Special Laboratory Design

BSL-3 and BSL-4 Laboratory and Animal Facilities

When the 1978 *Laboratory Safety Monograph*[1] was published, the term "Physical Containment", or P level, was used to indicate the type of facility design and engineering controls that were necessary to control the exposure of personnel to the accidental release of potentially hazardous agents within the laboratory. The *Monograph* provided physical containment guidance for P1 and P2 level recombinant DNA research that could be conducted in a conventional laboratory facility that did not require special design considerations. Experiments requiring P3 or P4 physical containment had to be conducted in facilities which met certain minimum design requirements specified in the "Special Laboratory Design" section of the *Monograph*. Since the publication of the *Monograph*, the terminology for biocontainment has evolved. The current terminology, biosafety levels or "BSL", promulgated in the CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL)[2] is universally accepted and has replaced the 'P level' defined in the *Monograph*. The various biosafety levels (BSL 1-4) are based both on physical containment requirements and the safe standard operating procedures (SOPs) developed for a specific laboratory. Guidelines are provided for minimum design requirements.

Currently, there are no uniform standards, regulations or measurable tests for high containment facility systems. Design professionals have attempted to define design and test criteria for containment test methods; regulatory authorities have relied on risk assessments and performance based objectives that define preferred outcomes without establishing methods to achieve expectations. Although documents such as the BMBL 5th edition,[2] the National Institute of Health's NIH Design Requirements Manual for Biomedical Laboratories and Animal Research Facilities (DRM)[3], American Society of

Heating, Refrigerating and air Conditioning Engineers (ASHRAE) standards,[4] U.S. Department of Agriculture Animal Research Service (USDA ARS) 242.1 Manual,[5] World Health Organization (WHO) Laboratory Biosafety Manual, 3rd edition[6,7] that are used as guidance to design, construct and verify BSL-3 facilities, they are often non-prescriptive and leave room for interpretation in the application of the guidelines. Many of these guidelines offer design requirements, but lack the testing and performance verification methodology to assure the safe operation of critical systems for these laboratories.

An extensive 'Gap Analysis' was conducted to determine what regulations, standards and guidance are currently available that provide a 'methodology' to verify that systems in high containment facilities are performing appropriately for their current or potential future use. Although some specific requirements and component testing procedures may be found in some documents (e.g., ANSI Z9.5, NSF 49), there is no single source for comprehensive methodologies that can be used to perform a risk assessment of each individual facility. The industry needs a more extensive graduated, risk-based approach to reaching containment goals appropriate to the risk of the agent and the laboratory activity.[8]

The Government Accountability Office (GAO) notes that a clear and unambiguous set of standards stating the various capabilities that are required to maintain the integrity of all high-containment laboratories is necessary.[9] Such a set of standards will need to integrate building codes with the BMBL provisions or amendments thereto. These standards should be national – not subject to local interpretation – and address the possibility that one or more emergency or backup systems may fail. Most importantly, any set of scenarios aimed at maintaining containment integrity must be empirically evaluated to demonstrate its effectiveness.

Peer reviewed publications by renowned bioenvironmental engineers are filled with the consequences resulting from the lack of uniformity in design, operation, commissioning and activation of BSL-3 facilities. The GAO report summarizes the complexity of the BSL-3 facility by stating that effective biosafety involves layers of containment; that the loss of any one layer is serious even though the remaining layers, as intended, do maintain containment.[9] Thus, procedures are required to regularly assess the functional integrity of every layer of

containment and to initiate immediate corrective actions as required. The fact that taken as a whole, containment is being maintained is not a sufficient measure of system integrity: each component must be individually assessed and its operational effectiveness validated on a regular schedule.

In light of the international efforts to establish high containment certification requirements and protocols for key components of high containment laboratories, and the application or use of ANSI standards as a basis for other international standards, it behooves us to establish a certification standard for BSL-3 facilities. This certification standard would not only fill the current void for BSL-3 laboratories, but would be a stepping stone to establishing certification standards for other types of containment laboratories, patient care facilities, airborne infection isolation units, and patient protective environments.

It is recommended that standards be developed that provide:

- Testing standardization, uniformity and consistency through the use of minimal performance based testing and verification methodologies for BSL-3/ABSL-3 systems.

- Technical background and information that addresses the engineering and associated systems within a BSL-3/ABSL-3 laboratory using the many principles of a risk assessment and performance based approach that is fully compatible with other bio-risk management systems, national and international health and safety management systems without duplicating or contradicting their requirements.
- Risk assessment guidance and methodologies to identify hazards that can be evaluated in terms of the likelihood that a problem may occur and the damage and/or consequences it would cause if such an event did occur.

- The collective knowledge of biosafety and design professionals and owners/operators who recognize the need to establish uniformity in the requirements and methodologies for the testing and performance verification of the BSL-3/ABSL-3 laboratory systems.

The National Institutes of Health has developed a Biosafety Level 3 Laboratory Certification Requirements and Checklist[10] as part of its Design Requirements Manual (DRM) 2008.[3] BSL-3/ABSL-3 facility certification is required for all NIH funded BSL-3/ABSL-3 laboratories and animal facilities and is highly recommended when NIH has grantee oversight responsibilities. The NIH certification document contains a comprehensive checklist of administrative and engineering controls required for certification of BSL-3/ABSL-3 laboratories. Many of the checklist items are based on NIH's experience with the numerous NIH funded laboratories currently being designed and constructed. An emphasis is placed on validation of appropriate standard operating procedures, protocols, training and maintenance of documentation for all regulatory compliance concerns, inspections and internal certifications (equipment, training, HVAC, etc.).

The American National Standards Institute/American Industrial Hygiene Association (ANSI/AIHA) Z9 Health and Safety Standards for Ventilation Systems is developing ANSI Standard Z9.14 "Testing and Performance Verification Methodologies for Ventilation Systems for Biosafety Level 3 (BSL-3) and Animal Biosafety Level 3 (ABSL-3) Facilities."[8] The design for BSL-3 ventilation systems has been largely guided by the criteria defined in successive versions of BMBL[2], ASHRAE standards[4], USDA ARS 242.1 Manual[5], WHO Biosafety Guidelines[6,7] and the NIH DRM[3]. Many of these guidelines offer design requirements, but lack the testing and performance verification methodology to assure the safe operation of the ventilation system for these laboratories.

Using a risk assessment and performance based approach, ANSI Z9.14 will provide the technical specifications and background information needed to address the technical, engineering and associated systems for ventilation within a BSL-3/ABSL-3 laboratory. It will be fully compatible with the comprehensive bio-risk management system which is covered by the CEN Working Agreement (CWA) 15793:2011[11] and other national and international health and safety management systems without duplicating or contradicting their requirements. The ANSI Z9.14 standard focuses specifically on ventilation system features of BSL-3/ABSL-3 facilities. ANSI Z9.14 will provide testing standardization, uniformity and consistency through the use of minimal performance based testing and verification methodologies for BSL-

3/ABSL-3 ventilation systems in facilities. ANSI Z9.14 is being designed as a 'one-stop' resource for the practitioner to use as guidance for inspecting and testing the performance of a high containment laboratory ventilation system when there is a change of laboratory function; change of agents to be used in the laboratory; in preparation for inspection by the Centers for Disease Control / Animal and Plant Health Inspection Service (CDC/APHIS), National Institute for Occupational Safety and Health (NIOSH), United States Department of Agriculture (USDA) or any other Authority Having Jurisdiction (AHJ); or in preparation for laboratory certification or on a regular frequency desired by the owner. It may also serve as a blueprint for future standards related to standardizing the oversight of other systems within high containment laboratories.

With the rapid expansion of high-containment laboratories, there is a desperate need for standardization and uniformity in all areas of the field to ensure that the facility is safe for occupants, the public, and the environment.

Biocontainment consulting and certification firms are emerging to meet the growing needs of biocontainment facilities. Unfortunately, there are no regulations to determine who is qualified and competent to certify biocontainment facilities. Therefore, provisions need to be made to provide training and accreditation for Biosafety Laboratory Certifiers.

Certification versus Accreditation

There is a distinct difference between certification and accreditation. Certification by an agency or organization is a "procedure by which a third party gives written assurance that a product, process or service conforms to specified requirements."[12] Accreditation is a "procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks."[12] The accreditation process should be carried out by a third party. Third party is defined as a person or body that is recognized as being independent of the parties involved, in this case independent of the laboratory or the laboratory's parent organization.

Several initiatives are currently under way nationally, internationally and at the institutional level to certify BSL-3 laboratories

and animal facilities. The NIH[3,10], the Canadian Food Inspection Agency and Health Canada[13], Ministry of Health Singapore[14] and Australian Government Department of Health[15], for example, have laboratory certification programs with BSL-3 specific requirements. There are several initiatives establishing an international approach to certification of laboratories such as the European Committee for Standardization CEN Working Agreement (CWA) 15793:2011.[11]

Laboratory certification is an on-going quality and safety assurance activity that should take place on a regular basis. Safety features that may be included in the certification process are the building and system integrity and examination of SOPs. This validation assures that all reasonable facility controls and prudent practices are in place to minimize, to the greatest extent possible, the risks associated with laboratory operations and the use of biohazardous materials.

Planning for certification of a BSL-3 facility should start during budgeting for the project and should be built into the design phase much as commissioning is planned for in the earliest stages of planning. If the designers and users are familiar with the checklist and testing requirements that will be used to certify the facility before it goes operational, then the certification process will be a much smoother process.

Laboratory verification/certification concentrates on three separate elements of the laboratory:

1. Biological Safety Compliance
2. Engineering Controls Compliance
3. Policies and Procedures (SOP) Review

The major recognized design guides (i.e., the BMBL 5th ed., NIH DRM, the US ARS Guidelines, WHO Guidelines), which are considered the "Standard of the Industry," outline the verification of the design and operational parameters for bio-containment laboratories. Failure to comply with the intent of these guidelines can be a legal and perceptual problem for the containment laboratory's owner institution. Currently, only laboratories that handle select agents are required to register with the federal government and receive certification that they comply with specified security regulations.

High containment laboratory certification helps ensure that:

- Appropriate site and protocol specific administrative controls and proper engineering controls are being used.

- Personal protective equipment (PPE) is appropriate and undergoes regular inspection to maintain personal safety for the tasks being performed.

- Decontamination systems for waste and other potentially infectious materials, including spill management, has been adequately considered and proper procedures are in place to mitigate environmental and personnel contamination.

- Proper SOPs for general laboratory safety and security, including physical, electrical, biological and chemical control mechanisms are in place.

A team of professionals with experience and credentials in engineering and biosafety/occupational safety and health should perform certification of high containment laboratories. This includes but is not limited to biosafety professionals and biosafety engineers with operational personnel, architects, construction personnel and the owner to ensure that the facility systems work in conjunction with the laboratory-specific work practices to establish integrity of the facility containment. When appropriate, the responsibility for providing certification of a laboratory or facility may be delegated to a third party.

Re-certification of the facility requires a comparison against the baseline established during initial certification. Therefore, detailed records of the certification process and test results must be maintained to provide an accurate operations history of the laboratory.

A standardized BSL-3 certification process for initial and annual laboratory certification would provide clear, unambiguous requirements that ensure the facilities physical integrity and the use of proper maintenance practices. Use of this process would demonstrate the use of SOPs that protect human and animal occupants, the environment and the research integrity for the numerous laboratories in the U.S. that

operate at the BSL-3 level. The certification process should include validation of and accounting for the completion of all other necessary and required processes or certifications such as applicable ASHRAE standards, NSF Biosafety Cabinet certifications, ANSI Fume Hood certification requirements, National Fire Protection Association (NFPA) requirements and local code and standard requirements. Certification should include evidence that a risk assessment for the generation of aerosols has been performed. A standard BSL-3 laboratory certification process would provide reassurance to the community that the laboratory in their neighborhood will in fact be operated safely and responsibly.[8] Verification/certification of bio-containment laboratories decreases the possibility of nuisance litigation and ensures the community that the laboratory can be operated at the highest standard.

American Biological Safety Association (ABSA)[16,17] currently manages programs for certification and registration of biosafety professionals but not facilities. ABSA is developing an accreditation program for the independent accreditation of high containment laboratories in the U.S. The accreditation process is using the CEN Workshop Agreement (CWA) as a guide for evaluating an organization's management system. Such an oversight process would assure lab workers and the community that a biocontainment facility has in place the necessary practices, procedures, personnel, and equipment to protect people, animals, plants, and the environment and minimize the potential of lab-acquired infections and lab accidents. Accreditation, conducted by an independent third party and using relevant national and international standards would be an effective way of ensuring competence in a comprehensive and uniform manner in laboratories working with biohazards. Typically, accreditation is voluntary. Key components assessed by an effective accreditation program would include: (1) the biosafety expertise and training of personnel managing and conducting the research; (2) the adequacy and function of the biosafety management structure supporting the research activities; and (3) the adequacy and function of biocontainment measures, including facilities, equipment, practices, and record-keeping systems, in place at the facility that is evaluated. ABSA has extensive experience in evaluating all three of these components. In addition, ABSA has established alliances with other groups that would provide support of this effort.

The BSL-3/ABSL-3 Facility

The BSL-3/ABSL-3 facility has special engineering features that make it possible for laboratory workers to handle indigenous or exotic agents that may cause serious or potentially lethal disease through inhalation route exposure.

Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents; they must be supervised by scientists competent in handling infectious agents; and they must be proficient in the associated procedures, use of personal protective equipment and the manipulation of infectious materials within Biological Safety Cabinets (BSC) and other physical containment devices.[2]

There is no "right" way to design a BSL-3 or ABSL-3 facility. The final design of the facility floor plan depends upon the needs of the program. Design professionals must meet with representatives from the administration of the facility, biosafety personnel, maintenance and operations personnel and end users to determine the type of facility that is desired. The facility must be designed in such a way that it is as flexible as possible and affordable for the institution. However flexibility is not infinite and no facility will meet the needs of every user all the time. Specific safety operations procedures can be developed to meet the containment requirements where some engineering controls may not have been installed. Since the publication of the *Monograph* in 1978, much has been learned about the engineering of biocontainment facilities. A number of new ideas have been implemented to ensure that there is minimal disruption of the containment integrity of these laboratories.

Meeting BMBL 5th Edition Criteria

The BMBL 5th edition provides guidance to maximize facility integrity and ensure the safety of personnel, animals and the environment. However, in some cases such as older facilities or facilities where an annual systems shutdown is not feasible, compliance to the BMBL can be met by performing and documenting that a risk assessment has been performed. For instance, the BMBL requires that in order to ensure that

the HVAC systems in BSL-3 and ABSL-3 laboratories meet the basic functional containment requirement during failure events, i.e., prevention of airflow reversal, these systems shall be tested before initial operation, and periodically thereafter as determined by the facility risk assessment and SOP and after any significant alterations of the ventilation system or other alterations that can affect it. The goal of any containment facility is to avoid airflow reversals of potentially contaminated air from containment spaces to "cleaner" areas. However, reason should be applied in light of the constraints of the facility and budget. Site-specific criteria related to reversals within the containment barrier may be stipulated in the risk assessment. For these reversals within the containment barrier, logically, criteria for sequences and events that occur more frequently (for instance a total power outage that might typically happen a few times a year) should be more stringent than for occurrences that are likely to happen much less frequently (for instance the tripping of a breaker that serves multiple exhaust fans). Site-specific acceptance criteria for higher risk laboratories should logically be more stringent than for lower risk laboratories. Acceptable responses of the HVAC system should be specifically detailed in the risk assessment for failures determined plausible. However, in no case should air originating in the laboratory or vivarium, escape through dirty corridors and anterooms to clean areas outside of the containment barrier.

Empirical testing of representative worst case excursions can be used to supplement the differential pressure data to demonstrate that air does not escape. Testing entities should conservatively apply engineering principles to demonstrate that air does not escape. If 'no reversal' / desired airflow is accomplished in empirical testing, a numerical testing methodology such as that proposed by[18] may be used to evaluate the potential risk. By using a numerical testing methodology, the amount of air displacement and contaminant leakage that might occur during a power outage that may result in a momentary positive pressure reversal in a BSL-3 facility can be calculated. The ultimate goal in design and operation of a BSL-3 facility is to achieve sustained directional airflow such that under failure conditions the airflow will not be reversed. The proposed methodology should be applied when and only when all other measures to achieve zero tolerance have been ruled out. Only after

determining that zero tolerance cannot be achieved for the BSL-3 facility in question should the numerical model be employed to perform a health and safety risk assessment to determine the reverse airflow tolerance.

The risk-based approach used in this example, may be applied to other instances when a facility is unable to meet the current version of the BMBL.

Sealing of Penetrations for Decontamination

While BSL-3 laboratories should be designed with the concept of directional airflow around the doors of the facility, all wall, ceiling and floor penetrations must be sealed with a smooth cleanable caulk seal. Such attention to the details of sealing penetrations is necessary for gaseous decontamination processes. Without such seals, the decontaminating gas could leak from the facility and not reach the necessary concentration for decontamination.

Pass-through Autoclave with Bioseal (preferred)

The *Monograph* recommended that an autoclave be present in the BSL-3/ABSL-3 facility but at a minimum an autoclave should be somewhere in the building. The BMBL 5th edition recommends that a method for decontaminating all laboratory wastes should be available in the facility but preferably within the laboratory.[2] Modern containment laboratories generally have pass-through autoclaves within the laboratory, or, at the very least, an autoclave on the same floor of the facility with very specific SOPs for transporting the waste from the BSL-3/ABSL-3 area to the autoclave. Where a pass-through autoclave is available, it should be installed with a bioseal to separate the containment side of the autoclave from the non-containment side. In addition, the autoclave body should be on the non-containment side of the bioseal to minimize heat load in the containment laboratory and to ensure easy maintenance of the autoclave. A local exhaust capture hood should be installed over the door of the autoclave on the non-contained side to capture any steam released from the autoclave at the end of the sterilization process. The exhaust duct from the autoclave capture hood must not be connected to the containment duct work as this would compromise the integrity of the

exhaust system. These autoclaves are not used for sterilization of materials going into the containment laboratory and therefore, do not require a capture hood on the containment side.

In the case of a malfunction of the autoclave or a temporary breakdown, the facility management must have SOPs for safe transport of waste materials from the containment laboratory to another autoclave in the building and insure that the back-up autoclave has been validated for treatment of the waste.

Electronic Door Interlocks into Containment Suites

While electronic door interlocks are not required for all containment laboratories, it has become industry standard to install them. Electronic door interlocks minimize the potential for more than one door to be open at a time. The installation of the electronic interlocks minimizes the potential escape of spilled material or infectious aerosols should a spill occur when both doors are open at the same time. When there are no electronic interlocks, a SOP must be developed to insure that no more than one door is opened at a time.

Dedicated, Redundant Utility Systems Separately Serving BSL-3 and ABSL-3 Biocontainment Areas

BSL-3/ABSL-3 laboratories may be involved in emergency verification of samples during a bioterrorism event and some biocontainment facilities may have to function during electrical outages. Therefore, they should be designed with sufficient utility redundancy of utilities to allow for continued performance of emergency tasks when necessary. There is a high cost to providing redundancy so where such emergency work is not required, redundant utility services may not be necessary. The decision to provide redundancy and how much redundancy for a specific facility should be made early in the design phase and it should be based upon the realistic verification of the scope of the laboratory work.

HEPA Filtration of Exhaust Air

Although the HEPA filtration of exhaust air from BSL-3/ABSL-3 facilities is still not required, it has become the industry standard and it is being provided in most new facilities. It is important to consider the ability to isolate, decontaminate, and test HEPA filter exhaust housings. The HEPA housings must have manual bubble tight dampers on both upstream and downstream sides of the housing to separate the housing from the HVAC system and allow for decontamination. Ports must be present for introduction of a decontaminating gas and for introduction of the test aerosol for leak testing. Some type of filter scanning device or protocol must also be available to provide for testing the filters for leaks.

Heating, Ventilation and Air Conditioning

The ventilation system supporting the containment facility must be capable of controlling air movement. The direction of airflow is to be from spaces of lower contamination potential to spaces of higher contamination potential. The system must be balanced so that there is infiltration of air into each laboratory module or animal room from the adjacent corridors. Doors must not be sealed during normal operation. It is recommended that the infiltration rate be in a range of 50 cubic feet per minute (cfm) to 150 cfm.

The 1978 *Monograph* states, "*The BSL-3 facility may be served by the same supply and exhaust air system that serves areas outside the BSL-3 facility, provided the exhaust air is not recirculated and air balance can be maintained. Air may be recirculated if the air is filtered by HEPA filters.*" Modern containment laboratories are generally designed with single pass air systems with no recirculation. Due to the requirement for directional air flow in these laboratories, the supply air to the labs should be dedicated, or capable of being completely shut down (fast acting bubble tight dampers) to minimize the potential for over-pressurization should an exhaust fan fail.

The exhaust air from BSL-3/ABSL-3 facilities is discharged to the outdoors clear of occupied buildings and supply air intakes. This is usually accomplished by locating the exhaust stacks on the roof and exhausting upward at relatively high velocity (e.g., >2500 fpm). The current requirement is for an exhaust upward velocity of 3000 feet per minute (fpm) (See NIH DRM Section 6.2.C - Location of Outdoor Air

Intake and Exhaust Air Discharge.[3]) The general exhaust air can be discharged to the outdoors without filtration or other treatment. However, in the majority of new containment laboratories, the exhaust from BSL-3/ABSL-3 containment labs is HEPA filtered prior to release to the atmosphere.

Each laboratory module of the BSL-3/ABSL-3 facility must be capable of accommodating a BSC. The treated cabinet exhaust air may be discharged directly to the laboratory module. In the latter case, it is important that the exhaust system be designed and operated in a manner that avoids interference with the air balance of the BSL-3/ABSL-3 facility and the BSC. Pressurization of the exhaust duct must be avoided.

Spare HVAC Capacity

Sustained directional airflow from areas of low containment to higher containment areas is critical to the maintenance of the containment integrity. Therefore, it is imperative that spare HVAC capacity is designed into the plan for the facility. Fans should be sized for a minimum of 20 percent excess capacity to allow for filter loading and potential for future expansion of the HVAC system. Redundant exhaust fans are also an important part of insuring the integrity of the containment facility. Although there are a number of different configurations of redundant fan operation, the most efficient and safe method is to have the two fans running simultaneously so that if one fan fails, the other fan ramps up to full speed and maintains the containment integrity. In this configuration, each fan must have sufficient capacity for maintaining the entire exhaust requirements for the portion of the facility that it serves.

Showers

Not all BSL-3/ABSL-3 facilities require a shower. The inclusion of showers in the BSL-3/ABSL-3 biocontainment design is dependent upon the type of work to be done, the agents used, and the risk assessment performed.

Enhanced Biosecurity

The requirement for increased biosecurity for BSL-3/ABSL-3 laboratories is a result of the increased concern regarding bioterrorism. Again, the early risk assessment with regard to the probability of potential incursion into the laboratory for the purposes of obtaining hazardous materials is necessary to insure appropriate biosecurity measures. When select agents, as defined by "The Public Health Security and Bioterrorism Preparedness and Response Act of 2002, Subtitle A of Public Law 107–188," are being used, appropriate biosecurity must be provided. It is important that the security devices and protocols enacted for each facility not compromise the containment integrity of that facility. Where possible, security cameras should be installed in such a way that they can be serviced from outside the facility. Security devices for entry and exit from the facility must allow for unobstructed egress from the facility in case of a life-threating emergency.

The BSL-3/ABSL-3 facility may be a single laboratory module, a complex of modules within a building or an entire building. The BSL-3/ABSL-3 facility is separated by a controlled access zone from areas open to the public and other laboratory persons who do not work within the BSL-3 facility.

Surfaces

The surface finishes of walls, floors, and ceilings should be resistant to liquid penetration and be readily cleanable. If windows are provided, they should be sealed shut in position. If false ceilings are installed to conceal air ducts and utility distribution lines, they should be constructed of plaster or drywall. All ceiling joints should be taped and sealed before the surface finish is applied.

The recommended floor surface is a monolithic-type covering that is free of seams or cracks and is coved to prevent potential leakage of spilled liquids. Openings in walls, floors and ceilings through which utility services and air ducts penetrate should be sealed to permit space decontamination. These openings can be effectively sealed by the application of a caulking compound. Acceptable caulking material can

be determined by reviewing the NIH DRM Exhibit X4-2-A, Sealant Table.[3]

Other Recommendations

A foot, elbow, or automatically operated hand washing facility should be provided near the exit area of each primary laboratory module. All doors of the BSL-3/ABSL-3 facility should be self-closing.

The BSL-4 Facility

As stated in the 1978 *Monograph*, the design objective of the BSL-4 facility is to create a facility that will allow the safe conduct of research involving biological agents that may present a high potential hazard to the laboratory worker, or that may cause serious epidemic disease. It should be noted that it is not possible to create a perfect design for a facility and "engineer out" the entire potential hazard from the operation of the facility. Research personnel must develop and follow safe protocols to minimize the potential of catastrophic spills that would put both themselves and the general public at risk to exposure.

The distinguishing characteristic of the BSL-4 facility is the provision of secondary barriers that prevent the escape of hazardous materials to the environment. The secondary barriers serve to isolate the laboratory area from the surrounding environment.

The secondary barriers include:

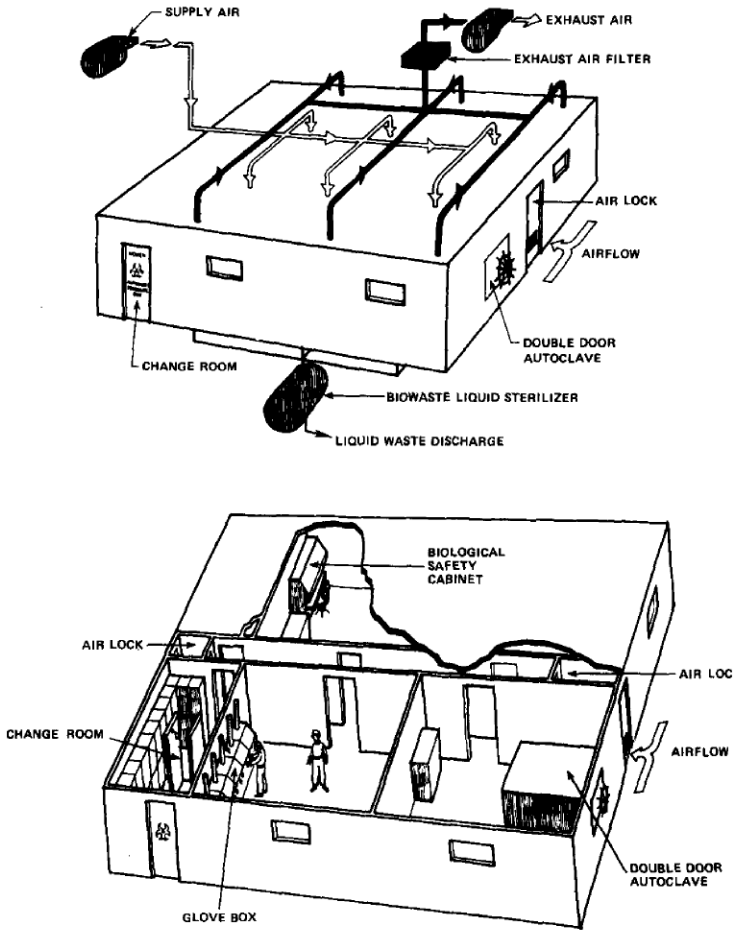
1. Monolithic walls, floors, and ceilings in which all penetrations such as air ducts, electrical conduits, and utility pipes, are sealed to ensure the physical isolation of the laboratory area.
2. Air locks through which supplies and materials can be brought safely into the facility.
3. Contiguous clothing change rooms and showers through which personnel enter the facility and exit from it.
4. Double-door autoclaves to sterilize and safely remove wastes and other materials from the facility.
5. Biowaste treatment (Effluent Decontamination System [EDS]) system to sterilize liquid wastes.

6. Separate ventilation system that maintains negative air pressures and directional airflow within the facility.
7. Treatment system to decontaminate exhaust air before dispersed into the atmosphere.

A comparison of the differences between the requirements and/or recommendations of the 1978 *Monograph* and the BMBL 5th edition for BSL-4 facilities is shown in the following table.

Facility Requirements

Figure 1. Secondary Barriers of the BSL-4 facility



SECONDARY BARRIERS IN REPRESENTATIVE P4 FACILITY

In this description of the requirements for BSL-4, there is no discussion of specific lab casework requirements, dunk tanks, or pass-thru boxes, hand wash sinks for cabinet labs, backflow prevention or lab vacuum

and HEPA filtration on vents, which are required at the BSL-4 level. These are covered in the BMBL 5th edition.

Suit Labs

The 1978 *Monograph* provided only the following information with regard to "Suit Labs":

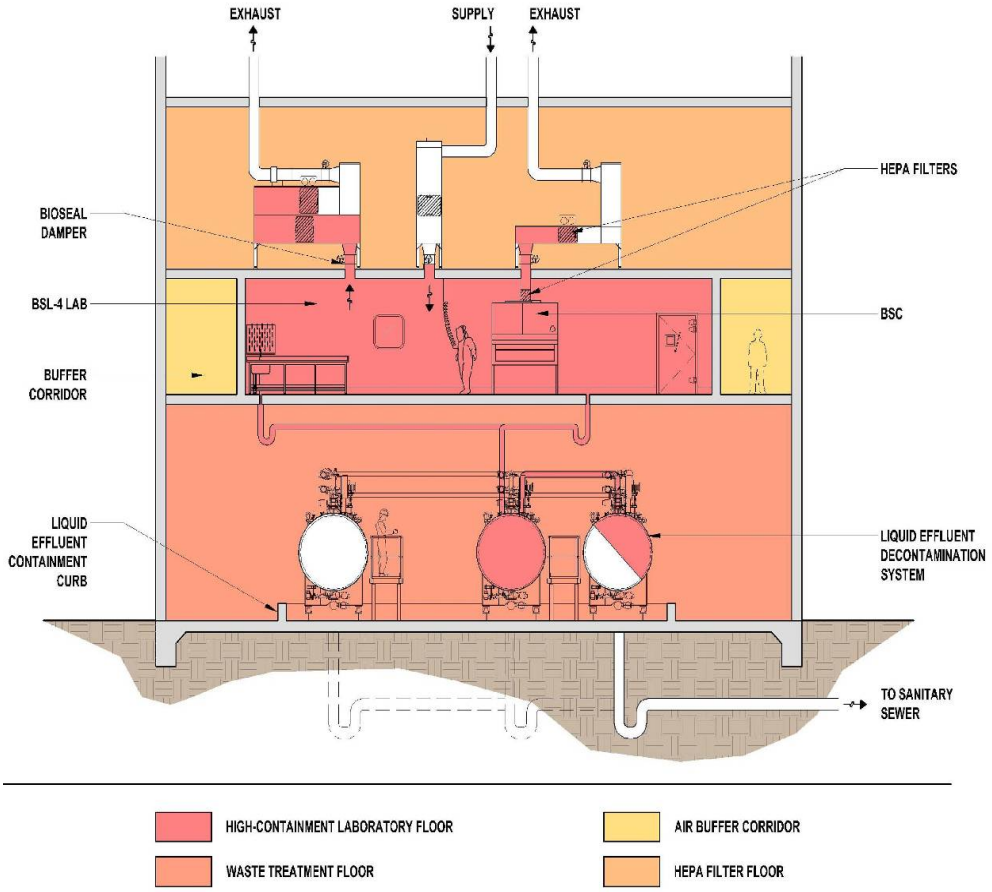
> "Primary protection may also be provided by having the laboratory worker wear a one-piece positive pressure suit while working in a specially designed suit area within the BSL-4 facility. The suit area is isolated from other areas of the BSL-4 facility by an air lock fitted with airtight doors, a double door autoclave, and a chemical disinfectant shower. The air pressure within the suit area is less than that in any adjacent area. The exhaust air from the suit area is separately filtered through two sets of HEPA filters installed in series, or filtered by a single HEPA filter, then incinerated before being discharged to the atmosphere. A duplicate filtration system and exhaust fan is provided. An emergency power source to operate the exhaust fans is also provided. The interior surfaces of the suit area have monolithic finishes, and all penetrations for utility services and air ducts through walls, floors and ceilings are sealed."

The *Monograph* indicated that the "Primary protection for the laboratory worker within the BSL-4 facility is provided by the use of Class III Biological Safety Cabinets." In the "cabinet lab" model, the primary containment is the Class III biosafety cabinet and all work is performed in that cabinet. Working in a Class III cabinet is difficult and designing the cabinet line that will have all the equipment and instruments necessary for a wide variety of research work is challenging. Since the publication of the *Monograph*, with the advent of more sophisticated control systems and instrumentation, the "cabinet lab" has been reduced in popularity and has been replaced, primarily, by "suit" labs.

In their 2011 presentation at the Safety by Design Symposium, Welter and Clinton emphasized that in designing and building a BSL-4 laboratory, there is a need to:

- Separate the facility from other non-containment areas
- Maintain pressure differentials/directional air flow
- Insure continual processing of effluent discharge during lab operations
- Ensure continual processing of potentially contaminated air
- Have HVAC interlocks and backflow prevention.

Figure 2. The Separation of the Containment Area from Other Non-containment Areas



Courtesy of Welter and Clinton, 2011

Figure 3. Cabinet Laboratory

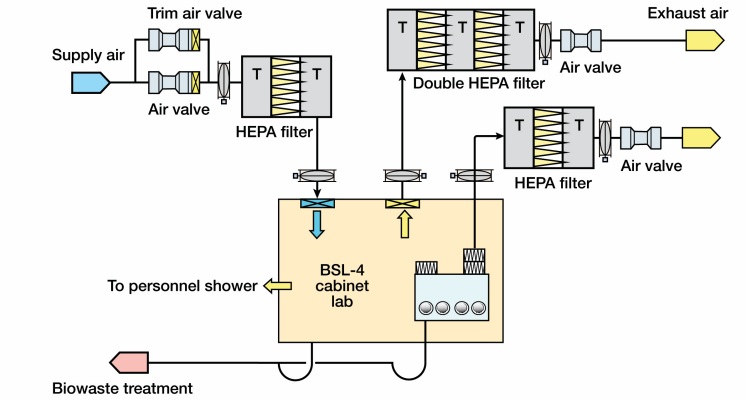
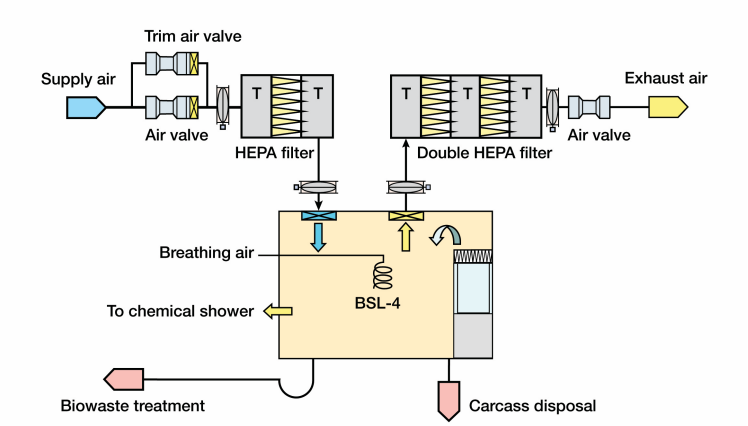


Figure 4. Suit Laboratory



Flexibility

In their presentations at the 2011 Safety by Design Symposium, Welter and Clinton, as well as Uri Yokel of Louviere, Stratton & Yokel, LLC, emphasized the need for flexibility in the design of these facilities, but one must remember that 'flexible' is not infinite. Often, in pursuit of flexibility, the design of these facilities becomes much more complex. The more complex the design, the more expensive it will be to build and the harder it will be to maintain and operate over time. Designers need to consider the ultimate costs and concerns of maintenance and operation of the facility when considering complex systems to solve simple problems. There will be times when, even though the facility is "state of the art," it cannot be used for a particular project because there is some inherent safety concern that cannot be met by either the engineering of the facility or a change in safety protocols. A realistic risk assessment must be performed to determine the potential hazards and the engineering requirements or safety protocols that will have to be used to insure personnel and environmental safety.

Welter and Clinton provided potential layouts of different laboratories for consideration at the Safety by Design Symposium as shown in Figures 5 and 6.

Figure 5.

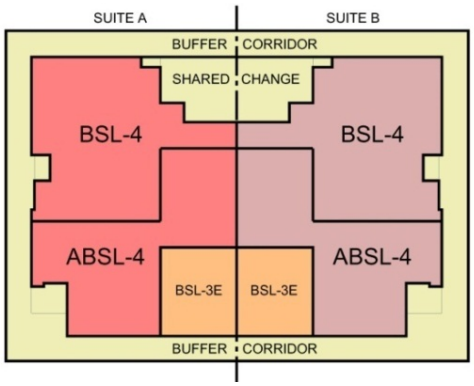
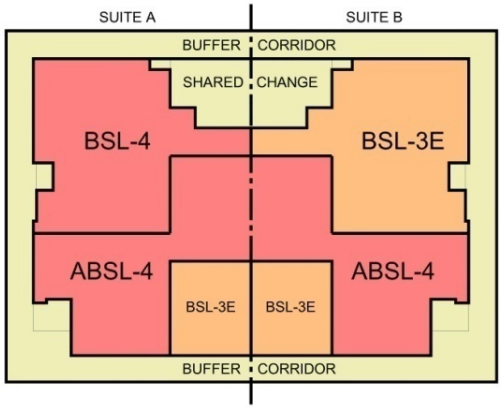


Figure 6.



Safety Considerations

Viewing windows or closed-circuit television should be considered to ensure that any emergency situation can be identified and appropriate actions can be initiated. Consideration of emergency egress and emergency decontamination showers is also important. Cameras and informational displays throughout the facility can provide information regarding potentially life-threatening problems within the facility to personnel outside of containment. Slip-resistant flooring in showers, necropsy areas, and animal rooms is also an important safety feature that should be considered.

BSL-4 suit labs must be designed with consideration for the potential damage to the suits, which protect the worker from possible exposure to the organisms with which they work. There is a need to minimize sharp edges and corners and to provide wide aisles for ease of circulation within the laboratory.

Decontamination

In the case of a catastrophic spill of infectious agents within the laboratory and outside of the primary containment device, the facility will have to be decontaminated. The decontamination agent of choice in

1978 was paraformaldehyde. The process for formaldehyde decontamination has been used for many years and has been demonstrated to be efficacious. However, the concern regarding the potential carcinogenicity of formaldehyde has initiated a review of other possible decontaminating agents. Both vaporized hydrogen peroxide and chlorine dioxide have been used to decontaminate potentially contaminated laboratories. In comparison to the paraformaldehyde process, these alternative decontamination methods are more costly and require more sophisticated equipment. The decision as to which method should be used in either BSL-3 or BSL-4 facilities must be made early in the design phase and should be based on a careful verification of the pros and cons of each methodology.

Risk Assessment

Throughout this *Biosafety Monograph*, we have mentioned the need to perform a risk assessment. Risk assessment is the qualitative and quantitative evaluation of risk posed to human health, animal health, and/or the environment by the actual or potential presence and/or use of specific hazardous biological agents or other materials. Risk assessment includes the exercise of identifying, analyzing, evaluating (probability versus consequence) and finally mitigating any potential hazard. A comprehensive risk assessment is an integral part of planning, design, construction, maintenance, and safe operation of any biocontainment facility and should be done prior to opening a new, or after modifications to an existing facility are made, and whenever agents or procedures change. Testing and verification of biomedical laboratory systems that operate at BSL-3 are necessary processes for ensuring that the performance and operation of the systems consistently maintain a safe environment for human occupants, research animals and the internal and external environment.

The risk assessment and subsequent Corrective Action Plan (CAP) will ensure that testing and validation procedures can be performed in a safe and secure manner for all personnel involved. An experienced team that includes personnel such as the biosafety officer, facility manager or director, veterinary staff (ABSL-3), building engineer, maintenance staff, and security personnel should perform the risk assessment. Since each

facility is unique, the risk assessment should be designed to factor in the specific features of each facility including but not limited to the containment boundaries, hazardous materials / biological agents, specific related SOPs including current decontamination SOPs, existing building engineering systems, existing system redundancies, and the facilities current maintenance program. It is highly encouraged that each facility develops and maintains SOPs that address testing and verification of the critical systems and associated components. Additionally, there should be SOPs for performing a risk assessment and for the 'Sequence of Testing and Performance Verification'. The risk assessment should be performed at three critical times in the life cycle of the facility: 1) opening a new facility, 2) after modifications to an existing facility, and 3) whenever agents or certain procedures change. Further, a site-specific risk assessment may also be performed at a defined frequency as determined by facility management.

"No amount of technological innovation can provide an absolutely safe facility. People are still the most critical part of the equation. Personnel must be trained not only in the research protocols that they will be performing, but also must be familiar with the design and operation of the safety features that have been built into the laboratory in which they are working. These personnel must also be held accountable for the proper operation of the facility."

– Farhad Memarzadeh, Ph.D., PE

The results of the risk assessment should be documented and maintained as part of the permanent record of the facility. The deficiencies that are identified in the risk assessment should be captured in a CAP that should be used in tracking remedial actions. Successive risk assessments should be performed until remedial actions are resolved.

Commissioning and Verification versus Certification

The 1978 *Monograph* noted that, "It is important that all mechanical systems and equipment of the facility are operating satisfactorily and

that appropriate maintenance is provided to insure continuous satisfactory operation." To this end, the *Monograph* provided detailed procedures, which were appropriate at the time, for testing facilities to insure that they met the containment requirements. The *Monograph* also stated that, "Adaptation or development of new procedures for certification is encouraged for situations where these procedures may not be applicable or best suited. A modified or new procedure would be acceptable provided it is capable of demonstrating that the criteria for certification are achieved."

A is defined by both the design and operational components of the containment facility. Containment facilities that support research at the BSL-3 and BSL-4 physical containment levels must provide certain facility "barrier" systems or safeguards that serve to protect persons and the environment outside of the laboratory setting from potential hazards associated with research. The appropriateness of a facility to support a specific biosafety level is, therefore, dependent on the performance of these facility safeguards.

Commissioning

Commissioning is a quality process for validating and documenting that a facility and its systems are planned, designed, installed, tested and capable of being operated and maintained to perform in conformity with the design intent. Commissioning begins with the planning phase of a given project and proceeds through design, construction, start-up, training, acceptance, and into early occupancy. The commissioning process is designed to ensure compliance with the design intent of the facility. Commissioning partially focuses on how the engineering controls comply with the overall building operations. When a building goes through the commissioning process, the applicable biosafety guidelines are not generally included in that process. The fundamental objectives of the commissioning process are:

- To create a SOP to verify and provide documentation that the performance of the equipment in the facility meet the design requirements;

- To enhance communication by documenting data and decisions throughout all phases of the project;
- To validate and report that building equipment performance meets the design intent.

A Commissioning Agent or Engineering Firm that is knowledgeable in building design and equipment operation and maintenance generally performs this work. Commissioning agents are often responsible for ensuring that the owner's maintenance personnel are properly trained in the operation and maintenance of the equipment that has been installed. Commissioning provides information on potential equipment deficiencies that can have an effect on a number of aspects of building operation including:

- Occupant comfort
- Energy efficiency
- Environmental conditions
- System and equipment function
- Building operation and maintenance

There are several concerns with the commissioning of the building/containment facility that impact on the final safe operation of the building from a containment standpoint. Since the commissioning process is designed to insure compliance with the design intent of the facility, if the design is flawed there may be problems with the actual containment integrity of the facility. Owners of proposed biocontainment facilities should always have the plans for the new facility reviewed by someone with experience in the actual operation of these facilities. While having very good engineering skills, most commissioning agents do not have laboratory work experience or a thorough understanding of laboratory work procedures. Reliance on only the engineering expertise can result in a false sense of security with regard to the containment integrity that depends not only on the engineering, but also on the facility procedures.

Verification / Certification

The function of a BSL-3 or BSL-4 biocontainment facility is to insure that a spill or aerosolization of an infectious agent within a laboratory does not escape the laboratory and cause harm to either personnel or the environment outside of that laboratory. Testing and evaluation of these laboratories should be performed on a regular schedule and the testing/evaluation process should document that the containment integrity will remain intact during any situation that may arise.

The BMBL 5th edition states, "The Biosafety Level 3 and 4 facility design and operational procedures must be documented. The facility must be tested for verification that the design and operational parameters have been met prior to the operation. Facilities should be re-verified, at least annually, against these procedures as modified by operational experience…Additional environmental protection should be considered if recommended by the agent summary statement, as determined by risk assessment, the site conditions or other applicable federal, state or local regulations."[2]

Once laboratories have been commissioned and begin operating, continuing maintenance and testing/validation programs are needed to ensure that operating standards and compliance to existing regulations and standards are maintained. Certification is the continuous process of validation and thus inherently different from commissioning. A comparison of commissioning versus biocontainment verification is shown in Table 2.

Table 2. A Comparison of Commissioning versus Biocontainment Verification

Action	Commissioning	Verification/ Certification
Testing of building systems	■	
Testing of Bio-containment laboratory systems	■	■
Verification of airflow and pressure differentials to ensure containment integrity	■	■
Review of preventive maintenance procedures and schedule	*	■
Administrative bio-containment laboratory policies and procedures reviewed		■
Containment envelope is checked to ensure appropriate sealing		■
Inspection to ensure that the bio-containment laboratory is cleanable		■
Provide site specific bio-containment training for personnel where applicable		■
Bio-containment laboratory certified to all applicable guidelines and regulations		■

* Commissioning agents may provide this test when instructed to do so.

As mentioned above, the 1978 *Monograph* provided guidance on the appropriate testing procedures for that time and stated, "Adaptation or development of new procedures for certification is encouraged for

situations where these procedures may not be applicable or best suited. A modified or new procedure would be acceptable provided it is capable of demonstrating that the criteria for certification are achieved." This advice is still valid today. The technologies have improved and with that, new procedures must be developed to document the efficacies of those technologies. It is important to realize that although the technology changes, the function of the biocontainment facility remains the same and the purpose of commissioning and verification is constant.

Since, by definition, a biosafety level is determined by a combination of SOPs and facility design, it is important to review the interaction of these parameters and ensure that the facility as designed and operated will retain its integrity at critical times. Verification/certification provides a measurement of that capability.

Specific testing and evaluation of the laboratories is dependent on the design of the facility and the equipment that has been installed. The key requirement is that the facility is designed and operated to minimize the potential for possible escape of the infectious agents that are being used and that the containment integrity is maintained at all times. Mechanical systems may fail, but quick action by personnel in following their prescribed emergency protocols will minimize the hazard of potential release. An annual evaluation of the containment facility with regard to the engineering controls and the procedural compliance is necessary to insure safe biocontainment facilities.

In summary, the basic principles of biosafety have not changed since 1978. New instrumentation and technologies have afforded both the design team and the facility personnel the capability of building and operating facilities that are inherently safer than the 1978 laboratories. However, no amount of technological innovation can provide an absolutely safe facility. People are still the most critical part of the equation. Personnel must be trained not only in the research protocols that they will be performing, but also must be familiar with the design and operation of the safety features that have been built into the laboratory in which they are working. These personnel must also be held accountable for the proper operation of the facility.

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Appendix A

Biohazards Symbol: Development of a Biological Hazards Warning Signal

From Baldwin, C.L. and Runkle, R.S. Science **158**: 264-265, 1967

Abstract. *The need for a symbol to warn of potential infection hazards became apparent during Public Health Service contract work on the development of containment facilities for virus - leukemia research. A program of direct inquiry and a search of the literature revealed that there was no universally used signal and that scientific and safety organizations concurred in the need for one. Criteria for symbol design were established, and final selection was based on "uniqueness" and "memorability." The National Institutes of Health is recommending use of the symbol as a warning of biological hazard.*

The Scientific community, engaged in infectious disease research, has accepted as unfortunate, but unavoidable, the occasional accidental infection of microbiology laboratory personnel and associated non-laboratory personnel. Since the mid-1940's, the seeming inevitability of such accidents has received an increasing amount of study. The eventual consensus was that perhaps most of these accidents need not happen, providing proper precautionary measures are taken and enforced. The last decade, in particular, saw great strides in the development of containment systems and in the design of safety equipment to protect the laboratory worker, his work, and the exterior environment from contamination by infectious agents. A new science of containment, founded on the concept of continuous agent control through the creation of intelligently designed barrier systems, has

emerged. Design of these barriers is based on a rational assessment of risk; the barriers may be created in the form of solid walls, pressure differentials to control movement of air, controlled movement of personnel and materials, or inactivation of the infectious agents themselves. In the maintenance of the barrier systems, one essential factor is that, at all times, the locations of the infectious agents must be known. In order not to inadvertently violate the barrier systems, each person in the vicinity must know what equipment, glassware, rooms, corridors, and ducts are contaminated by the infectious agents, and that thereby, they constitute a biological hazard.

Unfortunately, such biological hazards, like radiation hazards, are usually impossible to detect by cursory examination only. Being invisible, odorless, and tasteless, they require special procedures for detection. It seems logical, then, to mark the location of "biohazards," as they are commonly called, with a suitable warning sign that is readily noticed and easily recognized.

During investigations of biological control and containment conducted under contract for the National Cancer Institute, the need for such a symbol became apparent to the Dow biohazards research and development team. A search of the literature revealed that, while certain biological warning signs are used by various agencies, a *universal* symbol to warn of danger from infectious or potentially infectious agents – a symbol whose immediate significance is known to all – does not exist. Colleagues in the field of biological research concurred, in reply to direct query, that such a warning symbol is needed.

Universally accepted symbols for hazards that are not readily detectable have already been established, such as those used in denoting radioactive areas. Similar warning notices are being sought to point out danger due to laser emission. In biology laboratories, however, a number of different symbols are in use; none of these has been universally accepted, and none imply or encompass all possible biohazards. For example, an inverted blue triangle bearing the term "BIO" is used by the Army to warn of biological contamination; a rectangular "hot-pink" label, with radiating yellow bands is used by the U.S. Navy laboratories in areas containing infectious organisms; a red and black sign is used by the National Institutes of Health to mark restricted areas; and the white snake-and-staff imprint on a violet field is

sponsored by the Universal Postal Convention to make infectious materials during transit.

In formulating the design for the proposed biohazards symbol, six criteria were established, mainly dealing with the psychology of recognition and retention. These criteria, in order of their importance, are that the symbol be (i) striking in form in order to draw immediate attention; (ii) unique and unambiguous, in order not to be confused with symbols used for other purposes; (iii) quickly recognizable and easily recalled; (iv) easily stenciled; (iv) symmetrical, in order to appear identical from all angles of approach; and (vi) acceptable to groups of varying ethnic backgrounds. Dow artists created more than 40 symbol designs, of which six were selected for testing. A survey to ascertain acceptability of the six symbols was conducted among Dow employees. This survey was directed toward determining uniqueness and memorability.

To select the final symbol, a nationwide survey, based on precepts well established in mass-psychology experimentation, was conducted in two parts. First, the candidate symbols were tested for uniqueness by determining which had the least prior association for the viewer. Three hundred subjects, males and females, from 25 cities and with various amounts of income and formal education were shown the six symbols along with 18 other commonly used symbols. They were asked what each symbol meant, or was used for. Participants were also encouraged, if uncertain, to guess at the meaning. A “meaningfulness score” was obtained for each symbol based on the percentage of respondents who offered any association whatever, to the symbol. Since the purpose was to determine the least meaningful symbol, the smaller scores identified the most desirable symbols.

One week after the initial survey had been conducted, participants were revisited for a "memorability" test. The original respondents were shown a group of 60 symbols, which included the 24 seen during the first test. They were asked to identify those symbols, which they had been shown on the first interview. Each symbol was given a "memorability score" that depended on the percentage of participants who correctly identified the symbol as having appeared in the earlier test.

Identical memorability scores were obtained for two of the six test symbols, and these scores exceeded the average for the other 18 symbols

tested. Since one of the two also obtained the lowest score in the meaningfulness test, it emerged as the one symbol best qualified as being both unique and memorable.

Having evolved a suitable symbol, the next step was to attach the desired significance to it. It became important to define as clearly as possible how and under what circumstances the symbol should be used. A use standard was therefore prepared. This standard stipulates that the symbol "shall be used to signify the actual or potential presence of a biohazard and shall identify equipment, containers, rooms, materials, experimental animals, or combinations thereof which contain or are contaminated with viable hazardous agents." It also defines the term "biohazard," for the purpose of the standard, as being: "those infectious agents presenting a risk or potential risk to the well being of man, either directly through his infection or indirectly through disruption of his environment."

This symbol and the recommendations regarding usage have been submitted to the United State of America Standards Institute for inclusion in their next revision of the "Standard Specifications for Industrial Accident Prevention Signs," Z3S.1 code.

This symbol, in fluorescent fire-orange color, has been evaluated during a 6-month period at the National Cancer Institute and other selected laboratories engaged in studies involving hazardous agents. These cooperating research groups included the U.S. Army Biological Laboratories and U.S. Department of Agriculture laboratories, as well as a number of commercial and academic laboratories working under National Institutes of Health research grants and contracts.

In view of its acceptance by the scientists during this evaluation, the National Institutes of Health is recommending that this symbol be used as a general biological hazard warning.

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Appendix B

Origin of the 1978 NIH *Laboratory Safety Monograph*

The year 1969 was formidable for laying the foundation for the development of the National Cancer Institute (NIH) biosafety program and for promoting safe science in the conduct of research involving oncogenic viruses. President Richard Nixon terminated offensive biological warfare research, asked the U.S. Senate to ratify the 1925 Geneva Accord prohibiting the use of chemical and biological weapons, and signed an Executive Order outlawing offensive biological research. The Fort Detrick laboratory facilities, located in Frederick, Maryland, were transferred organizationally to the NCI and renamed the Frederick Cancer Research Facility (FCRC).

Construction of the first high containment laboratory on the NIH campus, built by the NCI to accelerate research for finding a human cancer virus, was also completed in 1969. Functional occupancy, however, was delayed for several years. High containment was required to ensure the protection of laboratory workers, facility support staff, and the public health from the potential risk of exposure to oncogenic viruses. The facility was built to meet the same design criteria used for microbiological facilities at Fort Detrick.

In 1972, the NIH published the *National Cancer Institute Standards of Biological Safety for Research Involving Oncogenic Viruses*. The standards increased awareness about the potential risks of cancer research and the importance of assessing those risks.

In January 1973, the NCI, the National Science Foundation, and the American Cancer Society sponsored a conference at the Asilomar Conference Center in Pacific Grove, California titled "Conference on Biohazards in Cancer Research." Two sessions of the Conference addressed potential biohazards associated with laboratory practices used in the typical cancer virus research laboratory, and methods to control biohazards in cancer research. Other presentations addressed the potential for accidental aerosol transmission in the biological laboratory;

hazards associated with the use of experimental animals, and precautions and facilities to minimize risk; and facilities and equipment available for virus containment. The Conference proceedings were published later that year.[1] The Conference attracted little attention, but kindled further thought. Also, in 1973, the NCI established the Office of Research Safety. The first priority of the Office was to address the potential hazards associated with handling oncogenic viruses.

In 1974, the NIH issued revised NCI safety standards, *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*. The new standards described three levels of risk associated with oncogenic viruses – low, moderate, and high – and provided criteria for assessing the potential risk of moderate and high-risk oncogenic viruses. The standards provided guidance on personnel responsibilities and practices, medical surveillance, laboratory and operational practices, and ventilated safety cabinets. For moderate risk laboratories, guidance for facility safeguards was limited to negative air pressure and directional airflow. For high-risk laboratories, guidance for facility safeguards also included air locks, personnel clothing change and shower rooms, and double door pass-through autoclaves.

In 1975, mounting concerns among scientists about the potential biohazards of the newly developed techniques for forming recombinant DNA molecules prompted the National Academy of Sciences to organize the "International Congress on Recombinant DNA Molecules." Held in February at the Asilomar Conference Center in Pacific Grove, California, this milestone by-invitation scientific meeting came known as "Asilomar." Risks and containment were fundamental issues discussed at the Conference. The Asilomar agreement described accepted principles in planning safe experiments, which were, "laboratory safety (containment) is an essential consideration in the experimental design" and "effectiveness of safety practices should match, as closely as possible, the estimated risk." The agreement described responsibilities of investigators, which included "risk assessment; inform laboratory staff of potential hazards; assure staff competency in safe practices; and, exercise considerable caution in performing experiments."

The NIH, under Director Donald S. Fredrickson, M.D., began developing guidelines for research with recombinant DNA molecules through the NIH Recombinant DNA Advisory Committee (RAC)

established in 1975. The first meeting of the RAC was on the day following Asilomar. The RAC accepted the general principles set forth at Asilomar, but at its second meeting, proposed more conservative guidelines for safe laboratory practices and containment than described at Asilomar. The first *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH Guidelines) was released on June 23, 1976, and published in the *Federal Register* on July 1976. The *NIH Guidelines* included Appendix D, which provided supplementary information on physical containment.

In August 1976, the United Kingdom issued *Recombinant DNA Guidelines*. The European Molecular Biology Organization (EMBO) found significant differences in the definitions and details of containment between the NIH and UK guidelines. The UK was not comfortable with the concept of biological containment. Although the differences in physical containment were minor, the differences concerned the NIH. In March 1977, Dr. John Tooze, Executive Director of EMBO, and I, then Director of the NCI Office of Research Safety, organized an international Workshop to find commonality in physical containment. The Workshop, "Parameters of Physical Containment,"[2] was held at the ARIEL Hotel in Heathrow, London, and included participants from laboratories in England, the United States, Germany, the Netherlands, Denmark, France, and Switzerland.

Commonality in physical containment was reached. The Workshop defined three levels of secondary containment provided by the use of facility safeguards: 1) the basic microbiological laboratory; 2) the containment laboratory; and 3) the maximum containment laboratory. The Workshop defined four levels of primary containment provided by the use of equipment safeguards, described as minimum, medium, high, and maximum.

On return to the United States, Dr. Fredrickson asked me to assemble a Special Committee of Safety and Health Experts to prepare the *Laboratory Safety Monograph* in response to numerous requests for greater specificity in describing practices, equipment, and facilities appropriate for the safe conduct of recombinant DNA research. The *Laboratory Safety Monograph* was published in July 1978 as a replacement for Appendix D and as a supplement to the first revision of the *NIH Guidelines.* The *Monograph* remains widely cited today.

The Preface of the 1978 *Laboratory Safety Monograph*, written by Dr. Fredrickson, reads in part:

> "The principal purpose of the 'Laboratory Safety Monograph' is to assist scientific institutions, principal investigators, and health and safety professionals in the selection and use of physical containment measures described in the revised 'NIH Guidelines for Recombinant DNA Research.' The information provided in this monograph is based on established principles of laboratory safety, expert opinion, and experience in dealing safely with infectious disease organisms in diagnostic and research laboratories. The monograph will be useful, therefore, not only to those associated with recombinant DNA research, but to all who are associated with research programs involving potentially hazardous organisms."

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[1] Biohazards in Biological Research - Proceedings of a Conference held at the Asilomar Conference Center, Pacific Grove, California, January 22-24; Edited by A. Hellman, National Cancer Institute; M. N. Oxman, Harvard Medical School; and R. Pollack, Cold Spring Harbor Laboratory. Copyright 1973 by Cold Spring Harbor Laboratory.

[2] *The Recombinant DNA Controversy – A Memoir – Science, Politics, and the Public Interest*. 1974-1981. Donald S. Fredrickson, M.D. Pg. 191. Copyright 2001. ASM Press. Washington, D.C.

Appendix C

Safety by Design Symposium Program

Plenary Sessions:

Laboratory Practices: Laboratory Techniques for Biohazard Control

Panel Moderator: Kelly Stefano Cole, Ph.D.
Associate Director, Regional Biocontainment Laboratory
Associate Professor, Department of Immunology
University of Pittsburgh

Presenter: Scott Aldermann, CBSP
Director of Operations
Regional Biocontainment Laboratory at Duke University

Presenter: Dee Zimmerman
Bioslafety Officer, University of Texas Medical Branch

Laboratory Practices: Decontamination and Disposal

Panel Moderator: Paul J. Meechan, Ph.D., RBP, CBSP
Director, Office of Safety, Health, and Environment
Centers for Disease Control and Prevention

Presenter: Lynne M. Sehulster, Ph.D., M(ASCP)
Health Scientist, Division of Healthcare Quality Promotion
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Centers for Disease Control and Prevention

Presenter: Joseph H. Wilson
Chief Executive Officer, Bio-response Solutions, Inc.

Laboratory Practices: Care and Use of Laboratory Animals

Panel Moderator: Joseph P. Kozlovac, MS, RBP, CBSP, SM-NRM
Agency Biosafety Officer, Animal Production and Protection
Agricultural Research Service, U.S. Department of Agriculture

Presenter: Keith E. Steele, DVM, Ph.D.
Senior Pathologist, Medimmune

Presenter: James R. Swearengen, DVM
Director of Comparative Medicine
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Containment Equipment

Panel Moderator: Jason E. Barr, MS, CIH, CDR USPHS
Environmental Health Officer
NIAID Integrated Research Facility – Frederick
Division of Occupational Health and Safety
Office of Research Services, National Institutes of Health

"Biological Safety Cabinets"
David C. Eagleson, BSE, MBA
President, The Baker Company, Inc.

"Certification Procedures"
James T. Wagner
President, Controlled Environment Consulting

Special Laboratory Design

Panel Moderators: Farhad Memarzadeh, Ph.D., PE
Director, Division of Technical Resources
Office of Research Facilities, National Institutes of Health
and
John H. Keene, Dr. PH, RBP, CBSP

President and Managing Partner
Global Biohazard Technologies, Inc.

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Uri Yokel, AIA
Principal, Louviere, Stratton & Yokel, LLC

"BSL-4 Laboratory and Animal facilities"
Alex Clinton, AIA
Senior Associate, Senior Project Manager, Perkins+Will
Jeffrey Welter
Principal, Science + Technology and
Higher Education Market Leader, Perkins+Will

"Laboratory Commissioning"
Glenn Dal Collo, CxA
Commissioning Field Coordinator, Merrick & Company

Operation Safe Science

Panel Moderator: Amy Wilkerson
Associate Vice President, Research Support
The Rockefeller University

"A University's Role in Promoting Safe Science"
Michael J. Imperiale, Ph.D.
Professor, Microbiology and Immunology
University of Michigan Medical School

"The Biosafety Professional / Responsible Official: Where Science, Security and Compliance Meet"
Joseph A. Kanabrocki, Ph.D., CBSP
Assistant Dean for Biosafety, Associate Professor of Microbiology
Biological Sciences Division, University of Chicago

Operation Safe Science

Panel Moderator: Robert J. Hawley, Ph.D., RBP, CBSP
Biosafety Consultant

"Emergency Procedures"
Wayne R. Thomann, Dr. PH
Director, Occupational and Environmental Safety
Duke University/Duke University Medical Center

"Medical Surveillance – Support and Response"
James M. Schmitt, M.D., MS
Medical Director, Occupational Medical Service
Division of Occupational Health and Safety
Office of Research Services, National Institutes of Health

Presentations

"Fostering a Culture of Biosafety and Responsibility in the Biomedical Research Laboratory"
Kelly Stefano Cole, Ph.D.
Associate Director, Regional Biocontainment Laboratory
Associate Professor, Department of Immunology
University of Pittsburgh

"Packaging and Shipping of Biohazardous Materials"
Christina Z. Thompson, MS, RBP, CBSP
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Thompson Biosafety, LLC

"Responsible Research in the Biological Sciences"
Rita R. Colwell, Ph.D.
Chairman of Canon US Life Sciences, Inc.
Distinguished University Professor
University of Maryland at College Park and
Johns Hopkins University Bloomberg School of Public Health

"Risk Assessment"
Stephen H. Hughes, Ph.D.

Director, HIV Drug Resistance Program
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National Cancer Institute, National Institutes of Health

"Safe Science in the Conduct of Research Involving High-risk Pathogens"
Carol D. Linden, Ph.D.
Principal Deputy Director, Biomedical Advanced Research and Development Authority
Office of the Assistant Secretary for Preparedness and Response
Department of Health and Human Services

"The Design of the Symbol"
Robert S. Runkle
Principal, Eagle Consultants

"The Ascendancy of Biosafety Training"
W. Emmett Barkley, Ph.D.
President, Proven Practices LLC

"Trade Offs in Microbial Threats Lists"
Arturo Casadevall, M.D., Ph.D.
Professor and Chair, Department of Microbiology and Immunology
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Work Groups and Moderators

Containment Equipment: Biological Safety Cabinets and Certification Procedures
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Laboratory Practices: Care and Use of Laboratory Animals
Joseph P. Kozlovac, MS, RBP, CBSP, SM-NRM

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Laboratory Practices: Decontamination and Disposal
Panel Moderator: Paul J. Meechan, Ph.D., RBP, CBSP
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Laboratory Practices: Laboratory Techniques for Biohazard Control
Kelly Stefano Cole, Ph.D.
Associate Director, Regional Biocontainment Laboratory
University of Pittsburgh

Operation Safe Science: Emergency Procedures; Medical Surveillance – Support and Response
Robert J. Hawley, Ph.D., RBP, CBSP
Biosafety Consultant

Operation Safe Science: Promoting Safe Science; The Biosafety Professional / Responsible Official
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Special Laboratory Design: BSL-3, BSL-4 Laboratory and Animal Facilities; Certification Procedures
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Appendix D

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