How you use our rack is up to you but we guarantee that each ISOcage will maintain a stable, lasting hermetic seal both on and off the rack. We also deliver each rack with a UPS battery back-up so it can be used independently of your power source.

Since safety is the #1 driver of bio-containment, our engineers designed HEPA filtration into this system at the cage-level. This makes personnel safety convenient, flexible and cost-effective since different studies can now be housed in the same rack and room.

We began by designing around the benefits of a premier biocontainment system that maintains negative pressure when the rack is undocked. Our engineers didn’t stop until we could offer you a better night’s sleep knowing that you made the most cost-effective choice in biocontainment.

Our ISOcage System comes to you with an unparalleled pedigree because each one is built with the insights and expertise of leading researchers and a world leader in equipment manufacturing.

The Tecniplast system combines an ISOcage of incomparable durability and design with a biosafety station offering the latest features in personnel protection.

Our system stands out—and it will stand the test of time—because our quality control measures are the most stringent in the industry.

The ISOcage System has all the elements of excellence that go into a Tecniplast product so you can be confident it has everything you need to keep your people and facility safe. To learn more visit us on the web at www.tecniplastusa.com or call 877-669-2243.

Looks like these guys got it right!
President’s Page

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**About the Cover**

Clinical microbiologists should handle cultures from sterile sites (spinal fluid, blood, middle ear fluid) in a biosafety cabinet. See Biosafety Tips (pages 47-49) for a discussion of this advice from the Centers for Disease Control to prevent exposures to *N. meningitidis* in clinical laboratories. The cover photo shows Arnold Steigerwalt, a research chemist with the Centers for Disease Control's Meningitis and Special Pathogens Branch (MSPB) in the National Center for Infectious Diseases (NCID), performing a DNA-DNA hybridization analysis in one of the CDC's laboratories.

The Meningitis and Special Pathogens Branch is responsible for monitoring an eclectic group of bacterial infections and disease syndromes of public health importance. The branch is organized into programs on meningitis, which focuses on *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*; vaccine-preventable diseases of childhood, which deals with *Bordetella pertussis* and *Corynebacterium diphtheriae*; zoonotic pathogens, which works with *Bacillus anthracis*, *Brucella* spp., *Leptospira*, and “non-tuberculosis” mycobacterial infections; and unexplained deaths and other emerging infections. Reprinted from the Public Health Image Library at www.cdc.gov; image #8406.
Vision

ABSA, the leader in the profession of biological safety.

Mission Statement

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

Goals

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

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Your Executive Council, Council, and ABSA Office continue to make strides for your organization. The weekly teleconferences with the Executive Council and ABSA Office, and the monthly teleconferences with Council Members, provide an effective means of communication. I have witnessed significant dedication and progress by your elected members and excellent management, direction, and support provided by your ABSA Office. I wish to share with you some of their progress and accomplishments during the past few months:

1. A revision of the ABSA Policies and Procedures document is reaching its final stage of review. This document describes the duties of each elected office member, expenses, meetings, committee appointments, and duties of Teams and the Team Leaders. This effort was lead by your President-Elect, Chris Thompson.

2. The Historical Committee, lead by your Council Member Joe Kozlovac, is pursuing an agreement with the U.S. Department of Agriculture (USDA) National Agricultural Library (NAL) to support the storage and maintenance of biosafety-related historical documents and equipment. For this collection, we are preparing electronic copies of photographs submitted by Manny Barbeito (formerly the National Program Officer for Biosafety at the USDA) and are awaiting a shipment of documents from Jerry Schmidt.

3. One mechanism to promote the discipline of biological safety and recognition of biosafety professionals worldwide involves registration as a biosafety professional (Registered Biosafety Professional, RBP). The Registration Committee, originally chaired by Rich Rebar, is now chaired by Betty Kupskay. We are working closely with ABSA members from the Former Soviet Union countries encouraging them to apply for RBP status. Translation of the RBP application documents and the relevant documents of potential registrants has been accomplished by Craig Reed and his support team. I wish all potential registrants success in their quest for recognition. The ABSA web site displays more of the registration process. Consider submitting your application for evaluation.

4. ABSA now has a Memorandum of Understanding with Sandia National Laboratories (SNL) that formalizes a partnership to enhance laboratory biosafety and biosecurity internationally. With this agreement, ABSA will provide biosafety expertise to support the international initiatives of SNL. Projects may include training, technical consultations, and an ABSA presence at regional conferences organized by SNL. Working through ABSA, Glenn Funk and David Bressler have participated in this endeavor with SNL during the past year.

Some other events you may be interested in include:

1. The Tradeline Conference, International Conference on Biocontainment Facilities, will be held in San Diego, California on March 12 and 13. ABSA will send two local ABSA members to represent our organization at this conference.

2. The Principles and Practices of Biosafety course will be held in Portland, Oregon during March 12-16. Presently, there are no spaces available for this course. I thank LouAnn Burnett for her work in promoting this effort.

3. The Winter Council Meeting will be held April 19-22 in Los Angeles, California. The Council and the ABSA Office will be exploring various hotels and meeting sites for the 2011 ABSA Conference. Our priorities include not only an acceptable venue, but also the availability of 100-200 hotels rooms within the U.S. Government per diem rate.

4. The Summer Seminar Series will be offered July 22-25 in Chicago, Illinois and will be held immediately preceding the Midwest Area Biosafety Network (MABioN) meeting. The Review Course for Biosafety for Exam in Biological Safety will be offered July 22-23 followed by two 2-day courses on July 24-25. There have been many requests to hold the review course earlier in the year to allow participants more time for review prior to the examination administered by the National Registry of Microbiology, American Society for Microbiology. Offering this review course in July will serve as a trial to gauge the interest in a summer offering.

5. Your Council has selected the Hyatt Hotel in Denver, Colorado as the site for the 2010 Biological Safety Conference scheduled for September 30 through October 6. We have negotiated 200 hotels rooms at the premium U.S. Government per diem rate (per diem plus 20%). This was the best option they could negotiate.

6. The ABSA Conference in October in Nashville, Tennessee will recognize this year as the 50th anniversary of the Biological Safety Conference. LouAnn Burnett and Dan Liberman are leading the efforts for this Conference. Plans are being considered to host a special presentation on the history of the profession (e.g., a Biosafety Roundtable). Invitees, recognized for their contributions to bio-
logical safety during the 1960s and subsequent years, will discuss some of their applied research and their experiences. These individuals have provided much of the foundation for what we practice and promote each day. Lodging will be at the Opryland Hotel and adjacent Radisson Hotel. A few years ago, your Council negotiated U.S. Government per diem rates for 40 rooms at the former and 75 rooms at the latter hotel.

Please let me know if you have any issues to be addressed, or if you wish to be involved with our many programs. I continuously seek your comments and suggestions. Also, I recommend you make your hotel reservations for the activities in Nashville as soon as possible and review the ABSA web site for additional information.

New Format for Posting Applied Biosafety in the “Members Only” Section of the ABSA Web Site

*Applied Biosafety: Journal of the American Biological Safety Association* (ISSN 1535-6760) is a peer-review scientific journal committed to promoting global biosafety awareness and best practices to prevent occupational exposures and adverse environmental impacts related to biohazardous releases. A goal of *Applied Biosafety* is to provide a forum to exchange and promote sound biosafety and biosecurity initiatives; publish new research in biosafety; provide information on best biosafety practices, policy issues and position papers; and provide a forum for biosafety and biosecurity information exchange to include editorials, commentaries, and reviews. We welcome and encourage submissions which further the profession of biosafety, biotechnology, research, medicine, and all related professions.

Do you need an article from an archived issue of *Applied Biosafety*? To make it easier to find, the web posting has been redesigned. Go to the “Members Only” section and click on an issue to bring up the table of contents. There is a link to each individual article. We hope this new, improved posting system will be helpful to members.

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Abstract

Designing a large-scale GMP production facility for biological production requires various types of risk assessments to be carried out. This is the main tool in obtaining a balance between the aspects where GMP and biosafety guidelines contradict each other. Only by evaluating the various risks involved in the project, can rational and optimal choices be made regarding facility design and construction.

Introduction

Defining biosafety and GMP is a first step toward understanding the similarities and differences in the approaches taken to attain safe working conditions and quality assurance in manufactured products. The definition of biosafety is: “a combination of procedures, containment systems, and construction technologies in order to minimize the risk of infecting laboratories and prevent escape of microbes into the surrounding environment.” The objective is to create a safe environment in which to research infectious diseases; to prevent escape of infectious agents, to minimize staff member’s and other people’s contact with infectious agents, both inside and outside the containment zone, and to prevent the introduction of infectious agents into nature.

Some biosafety guidelines take a performance approach. They define the intended result, not how to achieve it or how to demonstrate it. In this instance, the user develops and chooses the acceptance criteria. Other biosafety guidelines are more prescriptive. These outline specific requirements that must be met and, in some cases, they outline acceptance criteria as well. These guidelines are no doubt the most helpful in trying to convince i.e., the GMP authorities, that other interests are relevant as well.

The definition of Good Manufacturing Practices (GMP): “is the part of the quality assurance that ensures that pharmaceutical products are produced consistently and controlled in accordance with the appropriate quality standards. These standards depend on the intended use of the product and the requirements issued by the marketing authorization (MA) or the product specification. GMP applies to both production and quality control.” The purpose is not to keep the worker safe but to protect the end user of the product. While the guidelines for GMP production are different in Europe and the USA, they all focus on the end user and the actual requirements may vary a little.

Biosafety requirements must be considered with regard to the following issues:

• Manufacturers
  • Vaccine production
  • GMO (Genetically Modified Organisms)
• Hospitals/patient care facilities
  • Isolation rooms with or without airlocks
• Test laboratories
  • Vaccine production
  • Hospitals/patient care facilities
• Bioterrorism
• Animal facilities

However, when you consider manufacturers, it is relevant to address biocontainment and GMP at the same time. A case in point is that wild type polio virus is close to being eradicated worldwide and WHO has therefore published a guideline for production of Inactivated Polio Vaccine under BSL3 enhanced conditions. This is the first guideline that has tried to address both aspects.

Biosafety and GMP Synergies and Conflicts

It is easy to design facilities for GMP and biosafety containment when synergies are present. Synergies between GMP and biosafety guidelines include:

• Mandatory restricted access and segregation of production areas.
• Facility design should facilitate easy cleaning and assist in minimizing the introduction of airborne contaminants in the laboratory and production area.
• Validation of processes, systems equipment, and utilities must be performed.
• Job certification and mandatory training of employees must take place before work is begun. The training must be documented and repeated at regular intervals.
• Mandatory PPE (personal protective equipment) must be worn at all times while working with the agents and hazardous chemicals, etc. Training prior to the use of PPE is required, and written policies and procedures must be easily accessible.
• Tasks not documented are considered not done in a GMP environment. Documentation in biosecurity is essential and has become equally important in biosafety.

It is much more challenging to address the issues where GMP and normal biocontainment practices are in conflict with each other. Several of the more notable areas that demonstrate conflict between requirements merit discussion. First, those individuals unfamiliar with a large-scale GMP production facility should realize the rooms are very large in size as compared to rooms in a diagnostic laboratory, and have ventilation criteria similar to those required for animal facilities. Most of these production areas must be ventilated by up to 20 HEPA filtered air changes per hour, well in excess of standard BSL-2 and even BSL-3 laboratories. Second, part of the production area is sterile or aseptic and has no contact with the infectious agents, e.g., the initial cell propagating steps in the production of viral vaccines. To achieve this goal, these areas are stringently maintained under positive pressure relative to their surrounding corridors and laboratories. To further complicate matters, other parts of the manufacturing or developmental process involve work with infectious agents. The primary containment barrier in a production of biologicals is a fermentor. Vent filters are essential to ensure the virus is contained within the production vessel, and does not escape to other process areas. A relative or absolute negative pressure zone must be applied to these areas.

The design of the ventilation system is more complex than the traditional directional air flow as described for biocontainment laboratories, and the correct design and implementation is vital for achieving GMP status and producing products that are safe for human and animal use. Operation and maintenance of these systems pose ample challenges and costs, as there are many varied levels of pressure and air change requirements throughout the building. The secondary containment barrier in GMP as well as biosafety is the room itself.

When Worlds Collide: Conflicts Between GMP and Biosafety

Most major conflicts between GMP and biosafety occur in major systems areas such as facility layout, cleaning process flow, HVAC design, and decontamination/sterilization systems.

It is important to understand the reasons why GMP and biosafety practices are sometimes in conflict. GMP focuses on preventing cross contamination and keeping environmental contaminants out of the product, (Figure 1) thereby simultaneously protecting the end user and the product. In GMP, the production flow goes from dirty to clean. Raw materials entering the facility are considered dirty. The process includes several steps of purification and inactivation, which means the product becomes increasingly “clean” during the final steps of the production.

Biosafety focuses on keeping the infectious agent in, (Figure 1) thereby protecting the employees and the environment from possible leaks. The production flow is opposite that of a GMP production, i.e., clean to dirty or non-infectious to infectious. The production process be-
Dressed. It is important to make separate risk assessments and systems should also be considered and addressed. Incompatible interactions between these issues may involve: bubbles or foam, pH, redox, density, leakage, breakage, pressure, flow, volume, mixing, surface tension, creation of bubbles or foam, etc. In comparison to a small diagnostic laboratory, a facility engaged in the production of biologicals usually involves handling of large amounts of highly-infectious material. However, despite the large quantities, a topic that needs to be taken into consideration during the design phase, a normal GMP production usually only involves one type of infectious agent. It is important to understand that a biological production facility houses many tanks containing large volumes of product, waste, and growth media. The pipes inside the facility penetrate almost every room in the production area and carry liquids such as WFI (Water for Injection) at 80°C, deionized water, growth media, etc. If a pipe breaks during a spill, it will dilute the leaked material and almost certainly lead to the creation of an even larger volume of potentially-hazardous material that must be remediated. Aspects such as high pressure and temperatures are also issues that must be considered during large-scale production of biologicals, as compared to an ordinary biocontainment research lab.

Due to various aspects of GMP, the facility and systems design also includes closed systems, double filters, and steam traps on tanks, providing an extra level of protection to ensure the infectious agent stays within the tanks. Tube welders are used for inoculation or sampling. All systems are equipped with alarms and automatic shut down procedures. All handling is performed according to GMP procedures with batch records, GMP trained employees, SOPs, log books, etc. Finally, in addition to the described safeguards, the basic understanding between GMP personnel is: “that anything not documented on paper with the proper signatures, has not happened,” further ensuring proper operating procedures.

GMP or Biosafety: Which Guideline Wins?

GMP takes precedence at lower levels of biosafety risks (BSL-1/2), whereas biosafety takes precedence at higher biosafety risks (BSL-3/4). However, no compromises are acceptable in GMP production that might potentially increase the danger for the end user of the product. Both sets of guidelines must therefore be met when dealing with a large-scale GMP production and a high biosafety risk. Due to the responsibility to safeguard the end user of a product, standard technical solutions and basic design choices might have to be reconsidered. The following sections will provide examples of some conflicts to provide the reader a more detailed appreciation regarding what these issues may involve.

**Developing a Strategy for Merging GMP and Biosafety: Risk Assessment**

Merging GMP and containment aspects when synergy is not the case necessitates a strategy. As in all strategic planning, it is necessary to read all of the pertinent guidelines and to ensure you and those you will partner with understand them fully. Understanding why the guidelines and requirements differ is as important as understanding how they differ.

Alternate solutions to achieving a goal should be considered and discussed. One way to start is by reviewing the construction of similar facilities to learn how the issues were resolved in those particular cases. Risk assessment is a valuable tool in providing weighted values where there are contradictions between biosafety and GMP guidelines. While your team prepares a logical solution be aware that authorities governing licensing and approval may not be as familiar with the approach taken and the validity of the solution, and you will be required to defend your position.

A number of aspects must be taken into consideration while trying to establish the level of hazard associated with a particular agent. The following factors should be addressed in a risk assessment and thoroughly evaluated: reservoir, volume, concentration, possible ways of escape, route of transmission, infectious dose, susceptible hosts, incubation period, decontamination and whether immunization or treatment exists. It is important to remember that this part of the risk assessment is a subset of the total risk assessment which must be performed. For a large-scale production of biologicals, it is also relevant to perform a risk assessment on the mechanical performance of various production equipment and utilities. This part of the risk assessment highlights the most risky areas of a production by examining various possible scenarios.

Words such as: none, too much, too little, forgotten, more, less, part of, added, reversed, wrong direction, wrong component, wrong object, leaking, lost, too fast, too slow, too high, too low, too hard, too soft, too long, too short, too hot, too cold, etc. should be used to evaluate production equipment regarding temperature, pressure, flow, volume, mixing, surface tension, creation of bubbles or foam, pH, redox, density, leakage, breakage, tanks, pumps, valves, pipes, computer, alarms, communication, etc. Incompatible interactions between these issues and systems should also be considered and addressed. It is important to make separate risk assessments for normal production, plant shut down and restart for preventive maintenance, emergency or unplanned shut downs caused by, for example, fire or power failure or during CIP (Clean in Place) and SIP (Steam in Place) operations.
**Airlocks**

What is the best way for a door to open and how hard can it be to make that decision? (Figure 2). A higher pressure helps to keep a door closed, which means that in a GMP environment, it is normally preferred that all doors open toward the area with the highest pressure. However, seen from a biosafety point of view, all doors should open toward the largest room of the two, as this will create the smallest amount of air turbulence when doors are opened and closed. From a basic safety point of view, it is preferred that a door will not swing out into a corridor where people are expected to pass. From an emergency point of view, however, doors should always open away from areas where hazardous situations might occur.

A door can only open in one of two ways. This is fortunate as it means that at least some of the authorities will be satisfied by the end result.

How large should an airlock be? My experience is that most airlocks are too small, which means that the design does not allow room for all the equipment that needs to be installed in the area such as PPE, sinks for cleaning of hands, kits for handling of spills, emergency showers etc. Biosafety requires these items to be close by, while GMP specifies that they may not be stored or installed inside the production area, which means that storing them in the airlock might be the only option.

**Process Flow for Cleaning**

The process flow for cleaning must be decided very early in the programming phase. Both the GMP and biosafety guidelines specify that these flows must move from the clean areas to the dirty ones, and, in the case of GMP, the cleaning carts may not be stored permanently in the production rooms. Additional rooms for cleaning carts should be taken into account during the design phase, as it is impossible to add extra space for these later in the construction phase once the walls are constructed.

Sinks should be placed strategically to ease the drainage of water used during cleaning. Daily autoclave decontamination of the cleaning carts should also be considered in GMP. Consideration should be given to adding extra space to the decontamination area to enable storage of carts in case the autoclave is out of service for a short period of time. It is wise to plan to purchase an extra cart(s) as well.

**Ventilation Systems**

What about airflow? The airflow should be directed toward the containment zone, which must therefore be surrounded by another area with a higher pressure. This creates a pressure differential and an inward airflow. There are 3 ways to achieve this inward directional airflow (Figure 3):
- An absolute negative pressure within the containment zone is one option.
- Pressure may be neutral.
- A positive pressure within the containment zone is also an option, as long as a higher positive pressure is ensured within the rooms that surround the zone.

---

**Figure 2**
Which way should a door open?

![Diagram of airlock and doors]

- **Corridor**
- **Biosafety**
- **Virus**
- **GMP**
- **Emergency Exit**

+ **pa** - Positive pressure
Examining these 3 possibilities from both a biosafety and GMP perspective gives the following observations (Figure 4). From a biosafety point of view an absolute negative pressure is a very safe and effective design for a containment facility.

From a GMP point of view, however, this is a very creative and unusual way to ensure product safety. While the GMP guidelines do allow for this type design solution for a production of biologicals, and even though there are a lot of GMP inspectors, only a small number of them handle the inspections of biological production line or facility. Most GMP inspectors are accustomed only in visiting and assessing traditional pharmaceutical production facilities. A combined biosafety/GMP scenario is entirely new for them.

Neutral pressure is a compromise and as with most compromises, few individuals are cross trained to the degree that they understand and therefore accept a valid compromise.

Creating a directional inward airflow between two positive zones requires many biosafety professionals acting as inspectors to reassess the situation and think out-of-the-box. To many biosafety professionals, this is not a desirable solution or path, but to the GMP inspector this is an optimal scenario and they have a high level of understanding and comfort in accepting this solution.

Below, we will investigate these 3 scenarios:
An inward directional airflow must be maintained at all times no matter what happens elsewhere in the facility. This means that redundant ventilation aggregates in the
containment area are a necessity. This scenario is shown in Figure 5. If for some reason the grey fan should fail, containment will still be maintained.

In the next scenario shown in Figure 6: If the grey fan fails (6b) the pressure moves toward zero, which means that an inward directional airflow cannot be maintained. This can be rectified though, but only if there are redundant fans located outside the containment area as well (6c). Using this strategy will ensure an inward airflow.

The consequences of the last scenario (Figure 7) are more severe. If the grey fan fails (7b), the airflow will switch from an inward to outward airflow, which means that containment can no longer be maintained. The inward directional airflow can only be reestablished if redundant fans (7c) are available. Just as in the previous scenario.

There is no doubt that the first construction principle is the least expensive when assessing the ventilation cost. However, trying to run a clean room production with an absolute negative pressure is not an optimal scenario; it is more like a nightmare and raises the potential for costly failure.

The inward flow of particles from adjacent rooms in this scenario (through walls, doors, ceilings, and floors) has a heavy impact on the clean room status of the production rooms (Figure 8 “Unusual GMP”). It might prove very difficult to achieve a particle level low enough to ensure a safe product for the end user.

An air and particle tight room construction might therefore be preferred—just like a normal BSL-4 suit laboratory, however, in this case it is not to enable fumigation but to create a clean room environment.

**Figure 5**
First scenario, redundancy strategy.

![Figure 5](image)

**Figure 6**
Second scenario, redundancy strategy.

![Figure 6](image)
Liquid Effluent Decontamination System (AKA kill system)

This is not an area where the biosafety and GMP guidelines conflict, but building a liquid effluent decontamination system for GMP production adds some additional aspects that should also be taken into consideration as compared to a normal BSL laboratory or AG or animal BSL facility. A kill system for GMP production should be designed very carefully because, once again, more than one equity is at stake.

From a containment point of view, the kill system should be designed to handle large volumes of highly-concentrated virus harvest (and other infectious materials) when a full batch has to be discarded due to bacterial infection. Steam traps should therefore be installed above and beneath the tanks in order to be absolutely sure that the contaminated material will be kept inside the tanks, even if some of the valves develop a leak.

From a GMP point of view, the system should be designed “backwards” compared to normal systems seen in other BSL3 and BSL4 laboratory and animal facilities, meaning that the waste should flow directly into the treatment tanks. If a buffer tank is considered necessary, it should be placed after the treatment tanks—not prior to them. This enables the pipes leading toward the combined collection and treatment tanks to be steamed at regular intervals, ensuring that no bacterial infection can reach the production rooms through these pipes.

All collection pipes should be designed for routine

**Figure 7**
Third scenario, redundancy strategy.

**Figure 8**
Tight construction.
steaming and be drainable, from the production hall down into the basement in order to limit the risk of bacterial infection to the production area. Most of the waste running through these pipes is growth media, which means that any type of bacteria will be able to grow in them, and thereby potentially form biofilms that reach the production hall and contaminate the clean rooms.

Depending on the types of waste entering the kill system from the different production rooms (sometimes simultaneously) with other waste materials, the pH inside the treatment tanks prior to the heat treatment will be somewhere between 2-11. The waste inside the tanks is therefore pH adjusted before the heat treatment is initiated. The waste from cell production generates almost no solids. Grinding or homogenization is, therefore, not an issue.

It is also important to understand the types of waste that enter the plumbing system to the kill system. Sinks are used to drain water used during floors cleaning, the autoclave generates condensate, the CIP process (Clean in Place, carried out on all tanks) generates a lot of waste, with both nitric acid and sodium hydroxide. The upstream waste is mainly growth media, the SIP process (Steam in Place, of tanks with clean steam) generates waste in steam form, a waste that can lead to an increase in pressure or vacuum. The downstream process generates waste consisting of sodium hydroxide, acetic acid, saline, and acetate buffer. It is important to conduct all these types of waste down to the kill system through separate pipes into the manifold and further on into the tanks. The manifold should be designed as a backflow preventer—ensuring that a waste stream cannot go backwards up another pipe due to pressure differences.

**Design Responsibility**

Of course, there are many other issues to be considered before designing a facility or line for the GMP production of a product listed in a high-risk group. The topics described above represent a subset of examples of what should be considered before starting the initial programming phase. There are many additional issues that will have to be considered and discussed with an engineering company regarding the design of a new biological production facility. The chief of production is responsible for having considered synergies and possible conflicts. He or she is, in collaboration with the Biosafety Officer, also responsible for developing a thorough risk assessment (which includes addressing risk perceptions) and advocating the various design choices necessary. No engineering company or contractor, however skilled they may be, can make these decisions for the user. In the end, it is the facility manager that will have to defend and rationalize the design choices in front of both GMP inspectors, the biosafety professionals, and possibly the general public.

### Further Reading: GMP


The Rules Governing Medicinal Products in the European Community, Volume IV: Good Manufacturing Practice for Medicinal Products, Brussels.


**Further Reading: Biosafety**


World Health Organization. (1995). Biosafety Guidelines for Personnel Engaged in the Production of Vaccines and


Further Reading: GMP and Biocontainment


A novel emerging disease caused by the SARS coronavirus insidiously crept into the Asia Pacific region, beginning in November, in 2002 in Guangdong, China, then unrecognised as SARS. This was retrospectively diagnosed, and spread to Hong Kong, and from there to Vietnam, Singapore, Canada and Taiwan in February and March, 2003. Heroic efforts to contain the outbreak in each country led to these countries in turn being declared “SARS free” by the WHO. Taiwan being the last country infected, with the last known case having been detected on 15 June 2003, was declared SARS free in July 2003. However, this was only the end of the human to human transmission of SARS. What came next was a series of laboratory-acquired infections in laboratory staff; sequentially in Singapore (September, 2003), Taipei, Taiwan (December, 2003), and Beijing, China (December 2003 – January 2004). The genie had been let out of the box, inadvertently, and made these countries realize they had a laboratory biosafety crisis.

The events in the laboratory in Taiwan and Singapore have been submitted to this journal and will be presented in this quarter (Taiwan) and next quarter (Singapore). Events leading to the incidents and the significance of the events will be recounted. From the biosafety perspective, this was a unique opportunity to develop national and local biosafety standards with full support from the authorities. Together, they successfully worked to minimize the outbreak that not only led to health issues, but economic and emotional turmoil in the region. In all three countries affected, national standards or legislation has been implemented, and a flurry of activity to build high containment facilities has begun. These measures have gone hand-in-hand with training, communication, a determination to follow best procedures, transparent incident reporting, and good medical surveillance. There was a lack in many of these areas that led to the laboratory incidents. The lessons learned from these incidents, which happened in high containment laboratories (BSL-3 in Singapore and BSL-4 in Taiwan) only underline the importance of the human aspects of any good biosafety program. One can not rely on good engineering and facilities (although the Singapore laboratory was eventually downgraded to a BSL-2 laboratory). Retrospectively, it was indeed fortunate that in both cases the infections were confined to a single laboratory worker in question. This was not the case in the Beijing incident, where 13 cases were reported in total to have been connected to the use of a BSL-2 laboratory, with community spread. One other thing to note is that a BSL-2, BSL-3 and BSL-4 laboratory was involved in each case, and that every one of the laboratory staff involved in the incident were research workers. No diagnostic laboratory has reported any laboratory incidents involving SARS coronavirus. This shows that it is not so much the secondary engineering in the containment facility (though important), which is of paramount importance, but that proper biosafety management, and more stringent oversight, especially of the research community is required. The one good thing that came out of these unfortunate incidents is that lessons learned from them have bolstered national, local and individual efforts to improve the situation, and a much healthier biosafety culture will be the result. These incidents therefore have been chronicled to encourage other laboratories, not affected, to have a critical look at their biosafety culture and biosafety measures in place, and to do it in a timely way, to prevent more unnecessary incidents like these from occurring.
Abstract

A stunning laboratory-acquired SARS case broke out in Singapore in September 2003 and, only a couple of months later, a case also took place in Taiwan. The single infection case was diagnosed and confirmed, but did not spread, thanks to the swift inspection and emergency management of Taiwan CDC in December 2003. The CDC is Taiwan’s governing authority for prevention and control of communicable diseases, with full responsibility for management against severe biological hazards. Since this incident, we at Taiwan CDC have taken the opportunity to initiate an ongoing revision of the Communicable Disease Control Act and various regulations governing construction standards, occupational safety, fire security and environmental protection in Taiwan, in order to improve the standard requirements and mechanisms of supervising biological safety in laboratories. As a result, the level of biological safety of laboratories in Taiwan has been effectively enhanced through certain management measures of the government.

Through strict inspection of laboratory safety by CDC, along with education, promotion and practical training, all of Taiwan’s laboratories for microorganism examinations and related research have made great progress in terms of safety inspection of facilities and equipment, and awareness of safety and personal protection by the operational personnel. Aspects relating to inspection quality and research accomplishments, the physical safety of the research personnel and environmental security have been addressed and constitute a very meaningful and prominent indicator in the course of establishing Taiwan’s own laboratory management system concerning biological safety.

A National Biological Safety Committee was formed by relevant government institutions, in collaboration with several private organizations of biological safety for the purpose of policy integration, planning, promotion and implementation. Meanwhile, with complementary measures such as founding of a national information management system for laboratory microorganisms, establishing an accreditation system for laboratory safety, and enhancement in R&D in technology concerning domestic safety equipment, it is expected that the management system of laboratory biological safety would be comprehensive and enable Taiwan to become a meaningful and contributing member towards global biological safety.

Foreword

In December 2003, a laboratory-acquired severe acute respiratory syndrome (SARS) case broke out in Taiwan following on the heels of the first reported laboratory associated incident in Singapore. The Center for Disease Control of Taiwan (Taiwan CDC) immediately assembled an expert team to investigate (WHO, 2003) and found out that a laboratory operator who did not comply with management procedures on accidental spillage caused this single incidence. Fortunately, the affected person did not infect any one else.

One of Taiwan CDC’s predecessors, the National Institute of Preventive Medicine of Taiwan, obtained permission in writing from the United States CDC back in 1995 and translated their “Biosafety in Microbiological and Biomedical Laboratories, BMBL” (Third Edition) into Chinese, through which the concept of laboratory biological safety was introduced to Taiwan for the first time. Later, in 1999, our National Science Council established the Principles on Gene Recombination Experiments using guidelines set by the U.S. National Institute of Health as the primary reference, in order to provide protocols to laboratories for gene recombination (National Science Council, 1999). Each institution engaging in such research has to establish a Safety Committee on Biological Experiments to supervise, manage and examine safety concerns around the laboratory.

Similar to the organization of laboratory biosafety in developed countries in Europe and North America, our management of laboratory safety constitutes an area of occupational safety and health under the jurisdiction of the Council of Labor Affairs and other authorities regarding environmental protection and construction. The impact caused by Taiwan’s laboratory-acquired case of SARS...
in 2003 is significant. Taiwan’s CDC is the primary government authority to control communicable diseases with the full responsibility of preventing severe biological hazards. Thus, based upon professional opinions on health, medicine and microorganisms, a reform of the Communicable Disease Control was set into motion. Also, with legislations concerning issues of construction standards, occupational safety, fire fighting and environmental protection, taking a responsibility for improving and monitoring laboratory biological safety at the national level is deemed necessary. It is hoped that certain measures, such as autonomous management of laboratories, periodical inspection by external experts, and education to promote biological safety, will be helpful towards the establishing of a biosafety culture.

Since the accidental laboratory infection at the end of 2003, Taiwan CDC has initiated and implemented the following strategies and measures:

**To Facilitate International Exchanges**

In January 2004, the World Health Organization (WHO) dispatched an expert team led by Dr. Antony Della-Porta to Taiwan because of the SARS case (Figure 1). They visited five existing and new constructions of BSL-3 laboratories during their short stay. In addition to the advice given to those individual laboratories, four major measures in terms of general management of biological safety were suggested (WHO, 1993). Soon after, CDC invited two world-class experts in the field, Dr. Thomas Ksiazek from U.S. CDC and Dr. Kazuyoshi Sugiyma from National Institute of Infectious Diseases (NIID) of Japan, respectively, in March and November 2004, to give us a helping hand in inspecting current safety and management of equipment in laboratories of Biosafety Level 3 or above across Taiwan (Figure 2). CDC staff took notes on all drawbacks they found and suggestions they made (Taiwan Center for Disease Control Inspection Team, 2004) in order to assemble a set of guidelines for similar laboratories to incorporate. Also, Dr. Katsuaki Shinohara from NIID in Japan was invited to Taiwan in January 2005 to deliver a keynote speech on how to design and maintain the hardware facilities of BSL-3 laboratories to a group of engineers and laboratory operators. During the same trip, he visited eight newly con-

**Figure 1**
WHO expertise committee investigated BSL-3 virus laboratory of Taiwan CDC.

**Figure 2**
Taiwan CDC invited experts from USA CDC and Japan NIID to Taiwan to assist in appraising BSL-3 laboratories.

**Figure 3** (right)
Expert of Japan NIID invited to deliver a speech and assist in inspecting BSL-3 laboratories.
structured BSL-3 laboratories and provided valuable suggestions on improving hardware effectiveness (Figure 3).

To Establish Regulations on BSL-3 Laboratory Biological Safety

In response to the future needs of research and to monitor emerging or reemerging infectious diseases such as SARS, health authorities, medical institutions, agricultural administrations, the academia, and biotechnology companies are gradually building up BSL-3 or ABSL-3 laboratories. Presumably, Taiwan will have 21 laboratories at BSL-3 level or above by the end of 2005 (Table 1).

To encourage those responsible for the design and construction of laboratories to follow official guidelines, CDC invited local experts and scholars in September 2003 to write up a set of Biosafety Level 3 Laboratory Safety Guidelines (draft) and published it on the Taiwan CDC global information web (Taiwan CDC web site) for any interested person to download and criticize for further revision until the first edition was officially finalized in September 2004. The guidelines include organization management of BSL-3 laboratories, safety design of facilities, equipment function, inspection, personnel education and training, and internal inspection. In the future, revision and publication of newer editions will be carried out on a periodic basis to comply with the latest international rules.

To Strengthen Biological Safety Consciousness

After Singapore had its laboratory-acquired SARS case in September 2003, Taiwan CDC saw the coming danger and did try to contain the risk. It held seminars in November on both function test of BSL-3 laboratories and safety guidelines of BSL-3 laboratories in an effort to remind participants, who were domestic laboratory operators, of safety control and proper handling of various pieces of equipment in BSL-3 laboratories.

After Taiwan had a laboratory-acquired case in December, CDC immediately gave an order to suspend all laboratories handling the SARS virus across the island, and launched a special safety and response training course using the real scenario to educate the operators of BSL-3 laboratories in order to enable them to acquire practical experience. The trainees were asked to take an examination and only those who passed the test were awarded a certificate as officially qualified operators when the laboratory reopened for business.

Education and training at each laboratory level are aimed at the operation personnel, engineering and maintenance personnel, laboratory chiefs and biological safety officers to ensure their adequate knowledge about laboratory safety after 2005.

In addition, the CDC also did its best to enhance awareness of biological safety among staff of the examination divisions in medical institutions. All 517 medical institutions designated by the Bureau of National Health Insurance carried out a program of “boosting infection control.” To those institutions which carried out regular education and training on biological safety for their new and old staff members and with sound onsite management of pathogenic microorganisms, the Bureau would award a bonus toward their insurance claims in an effort towards emphasizing biological protection and safety to the examination personnel within medical institutions and reducing the potential of laboratory associated infection.

Legislation

While Taiwan CDC amended the Communicable Disease Control Act in January 2004, the concept of biological safety and preservation was included in its Article 32. Based on the Act, the establishment of a set of affiliated rules, i.e., Regulations Governing the Management of Infectious Biological Materials and the Collection for Testing Specimens of the Patients of Communicable Diseases was authorized. The regulations have been on the draft board since 2004. At its draft stage, CDC thoroughly tested the related measures to ensure good feasibility and applicability. With final approval of the Department of Health in August 2005, all 19 articles would be implemented after six months of promulgation period.

Highlights of the regulations are as follows:

1. To establish a management committee of biological safety

The committee of biological safety must be mission oriented that includes (i) to monitor and supervise maintenance, preservation, exchange and usage of infectious

<table>
<thead>
<tr>
<th>Biosafety Level of Laboratories</th>
<th>Before 2003</th>
<th>After 2003</th>
<th>Sum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSL-4</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BSL-3</td>
<td>3</td>
<td>15</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>ABSL-3</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

ABSL: Animal Biosafety Level
biological materials, which refer to risky microorganism groups of level two and above; (ii) to review biological safety level of a laboratory from time to time based on the infectious biological materials it deals with; (iii) to advise on handling infectious biological materials and on making improvements after drawbacks of laboratory biological safety are spotted by internal checks; (iv) to guide training for biological safety; (v) to review the plan in response to a biological safety emergency; (vi) to deal with, investigate, and report on accidents concerning biological safety; (vii) to assess laboratory inauguration and closure; and (viii) to appraise unsettled issues regarding biological safety. Through enforcing those missions of the biological safety committee of each unit, an ideal autonomous management will result.

2. Tightening up the qualification of laboratory workers

The regulation clearly stipulates that only those who have taken training from and passed the examination by the laboratory supervisor or senior technician are allowed to handle infectious biological materials in a laboratory operation. In addition, laboratory operators dealing with infectious biological materials of level three and above must take biological safety training duly certified by CDC before their laboratory operation proceedings. Through these stricter qualification measures, accidents due to lack of training could be avoided.

3. Making emergency plans to deal with biological hazards

Each and every unit must develop plans to follow in case of an emergency situation caused by biological hazards. It has to contain (i) an emergency response team with set missions; (ii) accident level certification and risk evaluation; (iii) procedures for accident handling, reporting mechanisms, and alarm systems; (iv) storage and management of materials needed in case of emergency; (v) procedures of medical aid for emergency; (vi) standards for personal safety and protection of personnel in the response team; (vii) procedures and measures for urgent evacuation; (viii) governance of damaged areas with means of clearance, integration, and resumption after disaster; and (ix) accident drills and scenario exercises. With such sound emergency plans in place, the unit can expect its personnel to follow correct procedures in case an accident occurs and thus minimize the damage.

4. The regulation of risk levels, managing and reporting on biological hazards

On top of clearly defining the risk levels in its description, managing and reporting procedures on biological hazards have been officially enacted. Taiwan divides biological hazards into three levels depending on the locality where they take place, i.e., (i) within a preliminary or first level protection (such as biological safety cabinets); (ii) within a second level of protection (such as laboratory facilities); (iii) with doubt of spreading into the environment. Regulations for managing and reporting are listed in Table 2.

5. Managing infectious biological materials

A set of specific regulations for managing infectious biological materials has been officially enacted. It consists of (i) storage sites of any infectious biological materials of level two and above should be put under the care and responsibility of a specific individual, with access control and inventories of level 2 and 3 risk agents; and (ii) use or exchange of any infectious biological materials of level three and above has to be reported to the central authorities in advance. Through strengthening security consciousness of infectious biological materials, we expect all highly infectious biological materials to be kept safely and securely.

6. The central authorities have the authority to order any facility to destroy certain assigned infectious biological materials singly or collectively within a time limit.

7. Regulations regarding import and export of infectious biological materials and testing specimens:

Any importation or exportation of infectious biological materials must apply for and obtain a letter of approval issued by the biological safety committee of the facility, which must then be sent to the central authorities for further approval.

8. Regulations for inspection of laboratories of biological safety level three and above, while they are in operation.

The central authorities can inspect those laboratories of level three or above at any time. Laboratories have a limited time to improve any shortcomings found during these inspections and the central authorities can shut down the operations of such laboratories if the required remedies are not completed within the prescribed time.

9. Inauguration guidelines for newly established biological safety laboratories, level three and above.

It is stipulated that any newly constructed biological safety laboratories of level three or above shall not commence operation prior to receiving approval from the unit’s biological safety committee and the CDC. (Table 3)

10. Regulations with regard to reopening of laboratories engaging in biological medicine and microorganism research and having been previously closed for any violation.

Whenever the central authorities have serious safety doubts, they could ask all laboratories or certain ones to stop using specific infectious biological materials or suspend the laboratory operation entirely. Resumption is allowed only after safety is ensured. The inaugurate procedures are shown in Table 4.

11. Regulations with respect to transportation and collection of infectious biological materials and testing specimens from patients of communicable diseases.

In 1999, one of CDC’s predecessors, the National Institute of Preventive Medicine, published a booklet written in Chinese called the Handbook of Specimens.
Collection for Disease Prevention, which was offered to all medical institutions and health bureaus in Taiwan to follow. The booklet was updated and revised in 2003, and its title was changed to the Handbook for Collecting Specimens of Infectious Diseases. In addition to the original standard operation procedures for the collection of all kinds of specimens, the following were added to it: (i) regulations concerning personnel safety and protection; (ii) rules of personnel physical checkup; and (iii) regulations on the cleaning and sterilization of work environment. They are there to ensure safety in collection and transportation of communicable disease specimens.

To Establish an Interministrial Biological Safety Committee for Policy Synchronization

Biological safety laboratories of all levels are affiliated to or under the jurisdiction of different ministries. For the sake of consistency in policy, Taiwan CDC specifically invited experts, scholars and representatives from the Ministry of National Defense, Ministry of Education, Bureau of Standards, Metrology and Inspection under the Ministry of Economic Affairs, National Science Council, Council of Agriculture, Environmental Protection Administration, and Council of Labor Affairs to found a permanent Biological Safety Committee in the Department of Health, under the Executive Yuan, in April 2004, which has convened regularly ever since. The Committee’s missions include: (1) to discuss and monitor management policies for biological safety at laboratories and workplaces; (2) to review and advise on management policies for biological materials; (3) to formulate and supervise the policies for transportation safety of biological materials; and (4) to communicate on the interministerial policies of biological safety, integration of resources and manpower and work sharing, as well as common problem solving. It is hoped these undertakings will establish horizontal channels of interconnection among domestic institutions and resolve conflicts of opinion.

To Plan and Organize Private Biological Safety Organizations

To assist the government in promoting policies and education on biological safety issues, biological safety associations have been established in the private sector in

| Table 2 |

Regulations of risk evaluation, management and reporting of biological laboratory accidents in Taiwan

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Incident Description</th>
<th>Management</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk Level 1</td>
<td>It occurs within protective equipments at laboratories, e.g., highly contagious specimens sprayed in biological safety cabinets during experimentation. The consequence does not endanger the lives of personnel immediately.</td>
<td>Handling following relevant hazard management procedures set forth in response plan to biologically threatening emergency.</td>
<td>Reporting to laboratory chiefs with records</td>
</tr>
<tr>
<td>Risk Level 2</td>
<td>It occurs outside protective equipments at laboratories, e.g., taking highly contagious specimens that cause spillage on the floor at laboratories; the consequence might endanger the lives of the personnel and contamination within laboratories.</td>
<td>Handling following relevant hazard management procedures set forth in response plan to biologically threatening emergency. If necessary, requesting assistance from relevant authorities is allowed.</td>
<td>Reporting to laboratory chiefs with records. Reporting to central authorities is required while the infection or contamination happens.</td>
</tr>
<tr>
<td>Risk Level 3</td>
<td>The spread happens outside laboratories. For instance, severe earthquake inactivates negative pressures at biological safety laboratories of level three. It might vitally endanger the lives of the personnel and contaminate the community and environment outside laboratories with hazards.</td>
<td>Handling following relevant hazard management procedures set forth in response plan to biologically threatening emergency. If necessary, the central authorities have to organize and lead other relevant units to manage the situation.</td>
<td>Reporting to laboratory chiefs with records. Reporting to central authorities is required at once.</td>
</tr>
</tbody>
</table>
Table 3
Inauguration procedures for newly established BSL-3 and above laboratories in Taiwan.

<table>
<thead>
<tr>
<th>The newly established BSL-3 and above laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>To complete establishment of software and hardware facilities</td>
</tr>
<tr>
<td>The function of laboratory equipment, biological safety cabinets and autoclave sterilizers are assessed to conform to standards.</td>
</tr>
<tr>
<td>With endorsement of biological safety committee within relevant division</td>
</tr>
<tr>
<td>To apply for onsite inspection by CDC</td>
</tr>
<tr>
<td>CDC organizes inspection team and to perform its duty</td>
</tr>
<tr>
<td>To achieve improvement based on inspection by laboratories</td>
</tr>
<tr>
<td>To apply to CDC for inauguration</td>
</tr>
<tr>
<td>CDC convenes assessment committee</td>
</tr>
<tr>
<td>Approval</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
</tbody>
</table>

Provide materials including:
1. Minutes regarding inauguration endorsement of biological safety committee
2. Plans for laboratory operation, training of management and emergency response
3. Reports on qualified inspection of laboratory facilities
4. Assessments on effective functioning of biological safety cabinets and autoclave sterilizers
5. Papers concerning standard operation procedures (SOPs)
developed countries, such as the United States, Japan, and Canada. Taiwan CDC cooperated with the Industrial Technology Research Institute and the Institute of Occupational Safety & Health in early 2005 in a joint effort to materialize the setup of a private organization, the Taiwanese Biological Safety Association (TBSA). It is the founders' hope that TBSA would (1) assist the government to develop policies for biological safety and health; (2) promote education regarding biological safety; (3) enhance the quality of domestic biological safety research; and (4) seek and facilitate international collaborations and exchanges of information regarding biological safety issues. In the future, TBSA is expected to develop useful and specific biological safety knowledge necessary for the nation, to help develop technology concerning biological safety, and to upgrade protection equipment and facility technology.

With the endorsement of the American Biological Safety Association (ABSA) in August 2005, TBSA became an affiliate member of ABSA. Being a member of the global village and, in such an era of advanced technology, Taiwan CDC believes actively participating in relevant international organizations and seminars is essential to acquire the latest information concerning biological safety and security as well as biological terrorism from other countries. We anticipate future attendance at the annual ABSA conference and active participation in education and training opportunities provided for members to keep us better informed on ongoing biological safety issues.

**Prospects for the Future**

1. **To set up a national information management system for laboratory microorganisms**

   In order to effectively oversee the current situation of management of infectious biological materials by laboratories at each level, Taiwan CDC began to construct a national system of information management for laboratory microorganisms in 2005. Through the system, goals to be achieved are (i) to keep notice of all events of storage, usage, exchange, shipping in and shipping out of any infectious biological materials of risk level 2 and above; (ii) to take note of the current situation of all domestic biological safety laboratories of level 3 and above in terms of their organization, personnel, and facilities; (iii) to provide Taiwan CDC with background information of those laboratories before inspection; and (iv) to bring about a nationwide operation platform for autonomous management of laboratories at each level.

2. **To formulate a suitable accreditation system for institutions engaging in inspection of BSL-3 laboratory safety**
So far Taiwan has no system of accreditation regarding inspection institutions of BSL-3 laboratory safety. Therefore, validity and credibility of inspection outcomes (such as laboratory facilities and security of biological safety cabinets) made by inspection institutions and companies cannot be officially recognized. Taiwan CDC plans to entrust the job to some widely recognized institutions of accreditation, such as the Taiwan Accreditation Foundation. Based on the international standard ISO/IEC 17020 (CNS 14725), a system of accreditation is to be established to enhance the technical competence and outcome quality of inspection institutions in order to achieve the goal of safety upkeep of laboratory equipment each year.

3. To upgrade the technical level of locally produced laboratory safety facilities and equipment

All BSL-3/4 laboratories that operated in Taiwan before 2003 were foreign made. Starting from 2004, almost all BSL-3 laboratories have been designed and built by domestic companies under the watchful eyes of experienced foreign advisors. With such a setup, we expect those domestic construction companies would gain the necessary expertise to become independent in the near future. Moreover, the quality of biological safety cabinets produced domestically is quite inconsistent due to lack of national standards. Therefore, in order to enhance the quality of biological safety cabinets made in Taiwan, the Institute of Occupational Safety & Health, Council of Labor Affairs and the Industrial Technology Research Institute will strive to institute some needed national standards and product specifications.

Conclusions

The laboratory-acquired case of SARS in Taiwan sent a shockwave through the nation. However, this incident was a crucial turning point. It led to the seeking and adoption of advice from both national and international experts and scholars and, therefore, acquired invaluable learning experience in biological safety management. This has facilitated the formulation of legislations on biological safety management and the implementation of related measures. Furthermore, founding a central interministerial Biological Safety Committee has helped develop effective horizontal channels of communication within the government. As a result, the undesirable egoism among government authorities could be minimized when promoting policies and regulations concerning biological safety. Furthermore, with the cooperation of private technical institutions and R&D institutes, a biological safety association has been formed to provide a platform of information exchange between industry, government and academia in the collective efforts to establish a management system of biological safety in Taiwan. It is expected that technical quality within biological safety industries would be enhanced and the personnel health and environmental security could be ensured. The importance of biological safety is no longer ignored with collective change. At the end, contributions to the global vision of biological safety would be made with active participation in international affairs of biological safety.

Acknowledgements

We are deeply indebted to our former CDC Director, Professor Ih-Jen Su (present Director of the Clinical Investigation of National Health Research Institutes) for his generous support and to Taiwan CDC, which regards the establishment and maintenance of a management system of laboratory biological safety as one of its major policies. Besides, the efforts and contributions of the participating ranks and files within CDC, as well as outside experts and scholars, are highly appreciated.

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Taiwan CDC Web Site: www.cdc.gov.tw


Animal Cell Cultures: Risk Assessment and Biosafety Recommendations

Katia Pauwels¹, Philippe Herman¹, Bernadette Van Vaerenbergh¹, Chuong Dai Do thi¹, Laura Berghmans¹, Geneviève Waeterloos¹, Dirk Van Bockstaele², Karoline Dorsch-Häsler³, and Myriam Sneyers¹

¹Scientific Institute of Public Health, Brussels, Belgium, ²Esoterix, Clinical Trials Services, Mechelen, Belgium, and ³Swiss Expert Committee for Biosafety, Bern, Switzerland

Abstract

During the last three decades, animal cell culturing has been essential for biomedical research and biotechnological activities in general. Along with this increasing importance, biosafety concerns have pointed to the risks of manipulating animal cell cultures for human health and the environment. A maximal reduction of these risks necessitates a thorough risk assessment of the cell cultures used. It involves an evaluation of both the intrinsic properties of the cell culture, including subsequent properties acquired as a result of genetic modification, and the possibility that the cell culture may inadvertently or deliberately become contaminated with pathogens. The latter is a major hazard associated with the manipulation of animal cell cultures, as adventitious agents may pathogenic and have a better capacity to survive in unfavorable conditions. Consequently, most of the containment measures primarily aim at protecting cells from adventitious contamination. Therefore, a comprehensive evaluation of the risks encountered during the handling of cell cultures should include considerations regarding the type of manipulation as well. As a rule, cell cultures known to harbor an infectious etiologic agent should be manipulated in compliance with containment measures recommended for the etiologic agent. With the exception of very well-characterized cell cultures for which the use of a type II biosafety cabinet depends on the origin of the cells, work with cell cultures from human or primate origin should generally and minimally be performed under containment level 2 using a type II biosafety cabinet. In every case, containment measures should minimize adventitious contamination of the cell cultures and offer a maximal protection of human health and the environment.

Keywords

Risk assessment, animal cell cultures, contained use

Introduction

The use of animal and human cell cultures has become very beneficial for diverse applications in the fields of biotechnology, medicine and veterinary. Originally used as substrates for the production of viral vaccines (Salk polio vaccine on monkey kidney cells, rabies, mumps and rubella vaccine using the WI-38 cell line), animal and human cell cultures became an indispensable tool to study intra- or intercellular responses and to serve as in vitro model for research. It has also been used for in vitro diagnosis of viruses or for the production of a wide range of biological products (hormones, interleukins, interferons and growth factors), including potential diagnostic and therapeutic products. More recently, cellular entity features associated to human and mammalian cell cultures have also gained interest in the search of new therapeutic approaches such as allo-/xeno- transplantation or cell-based gene therapy.

Along with the increasing importance of manipulating animal and human cell cultures in vitro, biosafety concerns have pointed to the risks with respect to human (as well as animal or plant) health and environmental considerations. A maximal reduction of these risks necessitates a thorough risk assessment of the given cell cultures, taking into account the type of manipulation and the implementation of appropriate containment measures.

Depending on the purpose or the type of activity, the use of animal cell cultures may fall within the scope of several regulatory provisions. In Europe, for example, as the manipulation of animal cell cultures may pose a risk related to the exposure of the worker to biological agents, this type of activity is covered by the European Directive 2000/54/EC. In many cases, tissue culture work will also involve the use of genetically-modified cell lines, in which case a risk assessment should be made in accordance with the provisions of the Directive 98/81/EC related to the contained use of genetically-modified organisms. Moreover, cell culturing activities aiming at manufacturing
biopharmaceuticals are covered by the Regulation (EC) No 726/2004 laying down procedures for the authorization and supervision of medicinal products for human and veterinary use, whereas activities that involve the use of human cells and tissues for application to the human body fall within the scope of the Directive 2004/23/EC which provides standards for the use of human cells and tissues in the category of cell therapy.

Since the use of cell cultures for therapeutic purposes addresses more extended considerations including quality, efficacy, safety, ethical, social and regulatory issues, it should be emphasized that the scope of this paper is limited to the risk assessment and risk management of diagnostic and research activities involving cell cultures. While biosafety recommendations (as outlined hereafter) are principally aimed at providing maximal protection of human health and environment, it is recognized that many of the precautionary measures would directly benefit the quality of research activities involving animal cell cultures as cross-contamination (Drexler et al., 1999) or inadvertent contamination with biologic agents are plaguing many researchers, often leading to inaccurate data, misinterpretation of results and a considerable waste of time and energy.

**Risk Assessment**

Risk assessment of animal cell cultures is based on both the intrinsic properties of the cell culture—including subsequent properties acquired as a result of genetic modification—and the possibility that the cell culture may inadvertently or deliberately become contaminated with pathogens. In addition, the risks encountered during handling of animal cell cultures should be evaluated with a careful consideration of the type of manipulation.

**Deliberate Infection of Cell Cultures**

Many biologic agents take advantage of a cell’s machinery in order to survive or to complete their life cycles. Therefore, the study of a pathogens’ life cycle or immunity escape mechanism may involve the deliberate infection of animal cell cultures. The determination of potential hazards related to infected cell cultures requires an examination of cell properties (as discussed below) and the inherent properties of the infecting pathogen. The latter implies an assessment of a number of criteria specific to the pathogen along with aspects such as the existence of effective therapies or prophylaxis. An evaluation of these criteria has been used to classify pathogens into classes of biological risk, also called Risk Groups. The four risk categories range from Risk Group 1, where a biologic agent is unlikely to cause human disease, to Risk Group 4, where the agent causes severe human disease and present serious hazard to workers with a potential of spreading to the community. Contrary to agents of lower Risk Groups, there are usually no effective prophylaxes or treatments available for Group 4 Biological Agents. Some reference lists originating from international authorities are covering natural biological agents (not genetically modified) as well as the group under which they are classified (2000/54/EC, Switzerland) or the biosafety level under which these should be manipulated (BMBL). As a rule, the biological risk of infected cell cultures will depend on the biological risk of the infecting pathogen(s). For example, cell cultures deliberately infected with Hepatitis C virus (HCV) in order to produce virus particles are assigned to risk group 3, as HCV is a risk group 3 viral pathogen. Nevertheless, as discussed below, the appropriate level of containment to be adopted will also depend on the type of manipulation. Another example is the infection of bovine leukocytes with Theileria parva, a tick-transmitted, intracellular protozoan of veterinary importance and the causative agent of East Coast fever among domestic livestock. It is an animal pathogen of risk group 3, which is not pathogenic to humans. The sporozoite form (infective form) invades bovine lymphocytes where it develops into a non-infective form (schizonts) and induces host cell transformation and clonal expansion of the cell. These infected bovine leukocytes may be categorized under risk group 2, while the biosafety level (BSL 1 or 2) appropriate for handling is determined by the presence or absence of the infectious form of the parasites.

**Adventitious Contamination of Cell Cultures**

Adventitious contamination of cell cultures is a major drawback for any activity that involves cell culturing (for a review see Langdon, 2004). In addition, one of the main biosafety concerns when manipulating animal cell cultures is the fact that animal cell cultures may provide a support for contaminating agents that cause harm to human health. Causative agents of cell contamination include bacteria, fungi, mycoplasms, parasites, viruses, prions and even other animal cells.

Generally, bacterial or fungal contamination can be readily detected because of their capacity to overgrow cell cultures. Typically, these organisms cause increased turbidity, pH shift of media (change in media color), slower growth of the cells and cell destruction. Antibiotics may be used to prevent cell contamination, however, continuous use of antibiotics in cultures may lead to development of resistant organisms with slow growing properties, which are much more difficult to detect by direct visual observation. Compared to bacterial or fungal infections, mycoplasma contamination gives more problems in terms of incidence, detectability, prevention and eradication. Mycoplasma, an intracellular bacterium, is one of the most common cell culture contaminants. It may go undetected for many passages and can change several cell properties such as growth, metabolism, morphology and genome structure (Paddenberg et al., 1996; McGarrity, 2004).
Animal Cell Cultures: Risk Assessment and Biosafety Recommendations

1985). It has also been reported to influence the yield of virus production in infected cells (Hargreaves et al., 1970). Hence, mycoplasmal contamination is also a biosafety concern, because some of the contaminating Mycoplasma spp. belong to risk group 2. Together with M. arginini, M. orale, M. pirum and M. fermentans, pathogenic organisms like M. gallisepticum (risk group 3 for animals), M. hyorhinis (risk group 2 for animals), M. pneumoniae and M. hominis (risk group 2 for humans) account for more than 96% of mycoplasma contaminants in cell cultures. Primary sources of contamination with M. orale, M. fermentans, and M. hominis in the laboratory are infected people who handle cell cultures and suspensions of viruses. Sources of M. arginini and M. hyorhinis are usually animal donors of tissues and biological constituents used for cell culture, e.g., calf serum and trypsin (Razin & Tully, 1995).

Viral contamination merits particular attention because infected cells may pose a serious harm to human health, especially when infected cells are able to release infectious particles. Human cells may be infected by various viruses like hepatitis viruses, retroviruses, herpes viruses or papilloma viruses. Although cell cultures from non-human origin may pose less risk, it should be emphasized that many viruses have a broad host range and can cross species barriers. Well-known viral contaminants of primate tissues or cells from non-human origin that can cause human disease are listed in Table 1. While contamination with some viruses may be associated with changes in cell morphology or behavior—such as the formation of syncytia (HIV, herpes viruses), swelling of cells (adenoviruses) or hemagglutination or haemadsorption—viral contamination may be harder to detect when cytopathic effects remain absent. Viral contamination could also trigger adverse effects as a result of recombination events or phenotypic mixing between contaminating components and experimentally-introduced agents, creating agents with new properties. For example, experimental results suggested that HTLV-I or HTLV-II undergo phenotypic mixing with HIV-1 in HTLV/HIV-1 co-infected cells, leading to an increase of the pathogenicity of HIV-1 by broadening the spectrum of its cellular tropism to CD4 negative cells (Lusso et al., 1990).

Adventitious contamination with parasites may be an issue when handling primary cell cultures or organ cultures originating from a donor organism that is known or suspected to be infected with a specific parasite. As the life cycle of most parasites comprises distinct developmental stages, transmission and survival of the parasite will strongly depend on the ability of the invasive stage to recognize and invade specific host cells. But even with cells developing the non-infectious form of parasites, possible harmful effects remain to be considered since natural modes of transmission could be bypassed during the manipulation of infected cells. It is recognized that most of the parasitic laboratory-acquired infections are caused by needle stick injuries (Herwaldt, 2001).

Finally, another class of agents that may contaminate cell cultures include unconventional agents that cause transmissible spongiform encephalopathies (TSE), the so-called prions (Solassol et al., 2003; Cronier et al., 2004; Vorberg et al., 2004). Neuroblastoma cell lines and primary cultured neurons and astrocytes have been shown to serve as hosts (Butler et al., 1988). Many studies have suggested that the risk of propagation of TSE agents in tissue culture cells, cultivated in the presence of bovine serum potentially contaminated with TSE, was restricted to neurons or brain-derived cell cultures. However, most recently, non-neuronal cells have been demonstrated to support TSE infection, suggesting that any cell line expressing normal host prion protein could have the potential to support propagation of TSE agents (Vilette et al., 2001; Vorberg et al., 2004). Contrary to most of the infectious agents, TSE agents are resistant to most of the physical and chemical methods commonly used for decontamination of infectious agents and may form a matter of concern in case bovine-derived products are used as tissue culture supplements.

Animal cell cultures can also harbor pathogens which are not known or whose tropism has not been defined yet. For example, viruses that were not known until recently include Hepatitis G (Linnen et al, 1996), HHV8 (Moore et al., 1996), TT virus (Nishizawa et al., 1997) or human pneumovirus (van den Hoogen et al., 2001).

Contamination of cell cultures is caused by different sources. Infected living organisms or infected cells or tissues from which a cell line has been established are the primary source of contamination. Contamination also occurs by the material used for cell culturing including glassware, storage bottles and pipettes due to improper maintenance or operation of sterilization of autoclaves. Presently, the use of disposable and sterile pipettes has considerably decreased the likelihood of adventitious contamination. In the past, the lip of the culture flask and the outside of the used pipette were found to be particularly heavily contaminated with mycoplasma agents (McGarrity et al., 1976). A third source of contamination resides in culture media and its components such as serum, basal cultural media, enzymes (trypsin, pronase and collagenase) and basic salt solutions. For example, media and additives derived from bovine sources are often contaminated with bovine viral diarrhea virus (BVDV) (Levings et al., 1991). Finally, air supply, clothing, personnel and floor can be a source of airborne contamination (Hay et al., 1991).

Genetic Modification of Animal Cell Cultures

The genetic engineering of animal cell cultures has benefited a number of applications in the biomedical and biotechnological fields. For instance, recombinant gene
expression in animal cells allows overproduction of biopharmaceuticals or biological products whose activity strongly depends on complex post-translational modifications of higher eukaryotic cells. Recombinant cells may also be chosen for their increased suitability or utility, i.e., for the replication of defective recombinant or wild type viruses.

The risk assessment of recombinant cells may be a complex task. Not only should properties that recombinant cells acquire following genetic modification be determined, an evaluation of each individual step in the process of genetic modification should be performed as well. This includes an evaluation of the recipient cell, the vector, the donor organism properties and an assessment of the characteristics of the inserted genetic material. The purpose of a biological risk assessment is to define biological hazards in order to be able to eliminate or prevent risks to both human health and the environment. However, despite the fact that cell cultures may harbor pathogens and pose serious biological risks to human health (as

### Table 1

Main viral contaminants of animal cell cultures or tissues that can cause human disease.

<table>
<thead>
<tr>
<th>Virus</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In Human tissues</strong></td>
<td></td>
</tr>
<tr>
<td>· Hepatitisviruses: HBV, HCV, HDV, HEV, HGV</td>
<td>Simmonds, P. (2001)</td>
</tr>
<tr>
<td>· Human Retroviruses: HIV-1, HIV-2, HTLV-1, HTLV-2</td>
<td>Popovic, M. et al. (1984); Clavel, F. et al., (1986); Poiesz, B. J. et al. (1980); Kalyanaraman, V. S. et al. (1982); Bhagavati, S. et al. (1988); Hjelle, B. et al. (1992)</td>
</tr>
<tr>
<td>· Herpesviruses: EBV, CMV, HHV-6, HSV-1, HSV-2</td>
<td>Whitley, R. J. et al. (2001)</td>
</tr>
<tr>
<td>· Papovaviruses: different HPV sequences, no virus production</td>
<td></td>
</tr>
<tr>
<td><strong>In Primate tissues</strong></td>
<td></td>
</tr>
<tr>
<td>· Flaviviruses: Yellow Fever virus, Kyasanu forest Virus</td>
<td>Tomori, O. et al. (2004)</td>
</tr>
<tr>
<td>· Simian hemorrhagic virus</td>
<td>Mahy, B. W. (1998)</td>
</tr>
<tr>
<td>· Hepatitis A virus</td>
<td>Dienstag, J. L. et al. (1976)</td>
</tr>
<tr>
<td>· Poliovirus</td>
<td></td>
</tr>
<tr>
<td>· Herpesviruses (Herpes B Virus and others)</td>
<td>Davidson, W. L. et al. (1960); Weigler, B. J. (1992); Hummeler, U. et al. (1959)</td>
</tr>
<tr>
<td>· SV40 (non pathogenic for humans)</td>
<td>Vilchez, R. A. et al. (2004); Dang-Tan, T. et al. (2004)</td>
</tr>
<tr>
<td>· Simian Immunodeficiency virus (infection but also disease in humans?)</td>
<td>Hahn, B. H. et al. (2000); Khabbaz, R. F. et al. (1994)</td>
</tr>
<tr>
<td>· Monkeypox</td>
<td>Likos, A. M. et al. (2005)</td>
</tr>
<tr>
<td>· Simian Foamy virus</td>
<td>CDC, Delelis, O. et al. (2004)</td>
</tr>
<tr>
<td><strong>In Rodent tissues</strong></td>
<td></td>
</tr>
<tr>
<td>· Lymphocytic Choriomeningitis virus (LCMV)</td>
<td>Mahy, B. W. et al. (1991); Hinman, A.R., et al. (1975); van der Zeijst, B. A. et al. (1983)</td>
</tr>
<tr>
<td>· Hantaan virus (hemorrhagic fever with renal syndrome)</td>
<td>Lloyd, G. et al. (1986); Mahy, B. W. (1998)</td>
</tr>
<tr>
<td>· Monkeypox</td>
<td>Likos, A. M. et al. (2005)</td>
</tr>
</tbody>
</table>
discussed above), it is unlikely that recombinant properties obtained by genetic modification may have an adverse effect upon release of the recombinant animal or human cells. Cells (genetically modified or not) have difficulties to survive in non-optimized conditions of growth, that is in a hostile environment where control of temperature and osmolality is lacking or where cell-specific nutrients (e.g., glucose, vitamins, lipids) are not balanced or missing. Therefore, independent of the possibility that genetic modification could confer an expanded life-span, immortalization or increased capacity for tumor induction, the survival of such primary cells or cell lines outside of proper conditions is unlikely to occur.

Recombinant cells are more likely to cause harm when entering the body of animals or humans. However, the extent of the harmful effect remains hard to predict. It should be kept in mind that the lack of histocompatibility between recombinant cells and the host organism remains a major obstacle for these cells to survive and to multiply as the natural immune response of the healthy (non-immunocompromised) host will recognize foreign cells and eventually destroy them. This is also one of the main reasons why the culturing of cells originating from the experimentor is not allowed for research and diagnostic activities.

Particular attention should be paid to the use of packaging cell lines. These are established cell lines which are deliberately and stably transfected with “helper constructs” to ensure the production of viral vectors. For example, in case of retroviral packaging cell lines, the expression of “helper genes” allows high-level constitutive production of viral proteins (e.g., gag, pol and env proteins), which are missing in the genome of the viral vector but are crucial for viral replication. One of the most important biosafety issues related to the use of packaging cell lines is the fact that replication competent viruses may be generated as a result of (homologous) recombination following transfection with viral vectors. Therefore the engineering of safer generations of retroviral packaging cell lines consists in minimizing the likelihood of generating replication-competent viruses by increasing the number of recombination events necessary to generate replication-competent viruses (Dull et al., 1998) or by reducing or eliminating the sequence homology between the viral vector and the helper sequences. However, endogenous retrovirus genomes expressed in safer generations of retroviral packaging cell lines may still give rise to unwanted recombination events (Chong et al., 1998). This means that the possibility to generate replication competent viruses is never completely ruled out.

Clearly, the risk group of the transfected packaging cell line will depend on the risk group of the retroviral vector itself. Consequently, risk assessment of packaging cell lines should be based on the biosafety of the produced viral vectors, including an evaluation of their infectivity, spectrum of host range, capacity of integration (insertional mutagenesis), stability and physiological role of the insert if expressed (for review see “Current Gene Therapy” Volume 3, Number 6).

**Intrinsic Properties of Cell Cultures**

Good knowledge and characterization of the intrinsic properties of cells constitute the key to successful and safe culturing. With respect to the biological risks related to the manipulation of animal cell cultures, three properties intrinsic to cell cultures should be considered specifically while performing risk assessments.

First, the species of origin should be taken into account. Based on the fact that pathogens usually have specific species barriers, the closer the genetic relationship of the cell culture is to humans, the higher the risk is to humans. The incidence to harbor organisms that could cause harm to human health is higher in human or primate cells in general, compared to cells of non-human origin (Brown et al., 1997). This means that mammalian cells (other than human or primate cells) are considered to represent fewer risks, followed by avian and invertebrate cells. However, it should be kept in mind that some infectious agents are able to cross the species barrier and to persist in new host species, leading to zoonotic diseases. Well-documented cases of viruses that have crossed the species barrier from animal reservoirs to humans include hantavirus (murine reservoir), haemorrhagic fever viruses (Ebola, Marburg) (Peters et al., 1992), avian Influenza virus and SARS-associated coronavirus (SARS-CoV).

Although increased growth, mobility and altered food patterns of the human population has been generally recognized as the main cause for the increased incidence of cross-species transfer of mammalian viruses, the occupational risks related to exposure to infected animal tissues or cell cultures should not be underestimated (Louv et al., 2005; Mahy et al., 2000).

Second, the cell type or type of tissue from which the cell lines are derived should be considered. Cell types dramatically differ in their in vivo half life: intestinal and certain white blood cells have a half-life of a few days, human red blood cells have approximately a 100-day half-life, and healthy liver cells rarely die, whereas, in adults, there is a slow loss of brain cells with little or no replacement. Partly due to this fact, some cell lines can be more readily obtained than others. The establishment of cell lines is often obtained by a series of (generally uncontrolled) mutations which occur by culturing cells for a longer period. It is a known fact that cells cultured for extensive periods of time display changing growth properties. A reduction of the doubling time, as a result of transformation, may give cells the ability to overgrow the rest of the population and to survive for a large (infinite) number of passages compared to primary cells with a finite life span. Therefore, the establishment of cell cul-
tures of a certain cell type upon extensive passage relies on the positive selection for cells that have a growth advantage. These transformed cells can have an increased tumorigenic potential and may present more risks of becoming or being fully neoplastic upon accidental or deliberate introduction into the human body. Therefore, taking the tumorigenic potential into account, the following cell types may be ranked in decreasing order of risk: hematogenous (e.g., blood, lymphoid) cells and tissue, neural tissues, endothelium, gut mucosa, epithelial and fibroblast cells.

For cell lines obtained from external sources (e.g., different laboratory), cross-contamination of cell lines, and/or a lack of proof of identity, is actually a widespread problem (Buehring et al., 2004). In order to have at least evidence of the species of origin of a cell line and to be able to conduct a thorough risk assessment, it may be necessary to characterize cell cultures. For this purpose, a number of techniques are available such as cyto genetic analysis, DNA fingerprinting, PCR, flow cytometry and isoenzymatic analysis.

Another inherent property to consider is the status of cell culture. Diagnostic and research activities involve the manipulation of primary cultures or cell lines, as well as continuous cell lines derived from primary cultures. Primary cell cultures and cell strains are produced directly from organs or tissues and are often the most accurate in vitro tool for reproducing typical cellular responses observed in vivo. However, as they are characterized by a finite life span, the time available for characterization and detection of contaminating agents remains limited. Also, because typical cell characteristics are often lost during the passage of cells, primary cell cultures are repeatedly obtained from fresh tissue, resulting in increasing risks for potential contaminating pathogens.

A feature that distinguishes continuous cell lines from primary cell cultures is the ability to survive if not infinitely, but for a great number of passages. These immortalized cells are obtained by isolating cells from tumors, by mutating primary cells with mutagens, by using viruses or recombinant DNA to generate indefinitely growing cells or by cell fusion of primary cells with a continuous cell line. Due to their increased life span, the time left for thorough characterization and detection of contaminating agents is considerably increased. Within this respect, well-characterized cell lines present the lowest risks compared to primary cultures or less characterized cell lines as the origin, the source and suitability are well-known and well-defined.

**Type of Manipulation**

Apart from assessing the properties of the cell culture, risk assessment related to handling cell cultures necessitates an evaluation of the type of manipulation, because processes, methods and/or equipment involved may increase or decrease the potential risks. For instance, though most of the established cell lines involved in large-scale operations are associated with low risks, the culturing of large volumes could give rise to new hazards due to inadequate containment measures. This may be the case for continuous processes such as cell cultivation in bioreactors where an appropriate design of seals, valves, pumps and transfer lines is required to guarantee long-term sterility of the operation. On the other hand, there may be less risk associated with the manipulation of "high risk" cell cultures or cells known to harbor infectious agents once they are fixed by glutaraldehyde or formaldehyde/acetone for immunostaining.

The following procedures and/or manipulations should be considered among research and diagnostic activities involving the manipulation of animal cell cultures:

- Procedures generating aerosols: pipetting, vortexing, centrifugation, opening of wet caps, etc.;
- Handling cells outside of a type II BSC: flow cytometric analysis and sorting of cell populations constitute a special case of cell manipulation in which cells are handled outside of a biosafety cabinet. The use of a fixative is, in many cases, not appropriate (e.g., viable cell sorting for subsequent further cell culturing) and the risk of aerosol formation can be particularly high, especially during sorting experiments and upon instrument failure such as a clogged sort nozzle. All scientists in the field of flow cytometry must be aware of the potential hazards associated with their discipline and only experienced and well-trained operators should perform potentially-biohazardous cell sorting. General recommendations approved by the International Society of Analytical Cytology should help to set a basis for biosafety guidelines in FCM laboratories (Schmid et al., 1997). Some procedures and methods have also been described for assuring the sorting of cell material under optimal biosafety conditions (Perfetto et al., 2004; Lennertz et al., 2005).
- Altering culture conditions: Changing the availability of cell-specific nutrients, growth factors, signal molecules or adopting co-culture techniques may have significant effects on handling of animal cell cultures as it may result in altered neoplasia (Stoker et al., 1990), altered expression of (proto) onco-genes or cell surface glycoproteins and release of endogenous viruses (Cunningham et al., 2004). As a consequence, changing culture conditions may lead to altered susceptibility of cultured cells to biological agents such as viruses (Anders et al., 2003; Vincent et al., 2004).
- Manipulations involving inevitable use of needles, sharps: due to injuries, cell material may be accidentally transferred directly to an operator’s tissue and/or blood stream.
- Animal studies: major risks are self-inoculation (needle-stick injury) and exposure to aerosols.

Finally, the purpose of cell culturing should be taken...
into consideration as many clinical approaches such as stem cell therapy, gene therapy, xeno- or allo-transplantation involve cell culturing ex vivo for therapeutic purposes. The latter clearly justifies more careful consideration regarding safety, ethical, social and regulatory issues, which cannot be addressed in this paper (FDA, EMEA, ICH, Stacey, 2005).

**Biosafety Recommendations and Containment Measures**

The examination of biological risks related to animal cell cultures and the type of manipulation allows the determination of a necessary and sufficient containment level in order to protect human health and environment. The set-up and implementation of an appropriate containment level include a list of general and more specific work practices and containment measures. Table 2 lists precautonary measures that should be applied whenever handling animal cell cultures. Many of these measures basically aim at reducing the risk of contamination with adventitious agents by ensuring protection of both operator and cell culture. It should be emphasized that the most important measure relies upon an adequate training of the operator. It should limit the possibility of contamination by the operator, benefiting the safety, as well as the productivity and quality of work.

As a general rule, cell cultures known to harbor an infectious etiologic agent should be manipulated in compliance with containment measures recommended for the etiologic agent. When cell cultures are not known to harbor infectious agents, cells may be considered free of contaminating pathogens as long as a number of conditions are fulfilled. This implies the use of well-characterized cell lines or controlled cell sources for primary cells such as specified pathogen-free (SPF) animals. If no well-characterized cell lines or SPF are available, tests for detection of likely contaminating agents should be negative. Second, whenever cell cultures are manipulated, media sources should be pathogen free and appropriate containment measures should be adopted to reduce potential contaminations during sampling or subsequent manipulation of cells (reefeeding and washing steps).

As the history of a cell culture may be poorly documented when a given cell culture is manipulated for the first time in the laboratory, it often remains unclear whether all appropriate measures have been implemented regardless of the fact that it may have been manipulated for years in another laboratory facility.

In this case, cell cultures should be considered to be potentially infectious and should be manipulated in a class II biosafety cabinet (BSC). If there is likelihood of the presence of adventitious agents of a higher risk group, the cell line should be handled under the appropriate containment level until tests have proven the absence of such organisms. Good documentation of the history of cell cultivation is mandatory.

The extent at which cell cultures should be con-

<table>
<thead>
<tr>
<th>Table 2</th>
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<tbody>
<tr>
<td>Precautionary measures for handling cell cultures*</td>
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<tr>
<td>- respect good microbiological practices, especially those that are aimed at avoiding accidental contamination.</td>
</tr>
<tr>
<td>- avoid opening of culture vessels or contact with culture fluid through a defective culture vessel, stopper or poor technique because of the ever-present likelihood of contamination with airborne pathogens.</td>
</tr>
<tr>
<td>- treat each new culture that is manipulated for the first time in the laboratory facility as potentially infectious.</td>
</tr>
<tr>
<td>- clean up any culture fluid spills immediately with a validated disinfectant.</td>
</tr>
<tr>
<td>- work with one cell line at a time and disinfect the work surfaces between two handlings involving cell lines.</td>
</tr>
<tr>
<td>- aliquot growth medium so that the same vessel is not used for more than one cell line.</td>
</tr>
<tr>
<td>- avoid pouring actions, which are a potential source of cross-contamination.</td>
</tr>
<tr>
<td>- proceed to an adequate use of the biosafety cabinet, this is turn on for a period before and after use, thoroughly disinfect BSC surfaces after each work session and do not clutter the BSC with materials.</td>
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<tr>
<td>- restrict the use of antibiotics in growth media.</td>
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<tr>
<td>- quarantine new cell cultures to a dedicated BSC or separate laboratory until the culture has been shown negative in appropriate tests.</td>
</tr>
<tr>
<td>- carry out a quality control of cells demonstrating the absence of likely contaminating pathogens on a regular basis or whenever necessary.</td>
</tr>
<tr>
<td>- handle cell cultures from undefined sources as risk group 2 agents. If there is a reasonable likelihood of adventitious agents of higher risk class, the cell line should be handled under appropriate containment level until tests have proven safety.</td>
</tr>
</tbody>
</table>

*Adapted from Doblhoff-Dier, O., Stacey, G., et al., 2000.
controlled on the likelihood of contaminants strongly depends on the nature of activity. The World Health Organization and regulatory authorities, both in United States and in Europe, have formulated guidelines designed to minimize any potential risk for transmission of infectious agents in case animal cell cultures are used for the industrial production of biological with therapeutic purposes (EMEA, FDA, ICH, WHO). It includes extensive testing of the cell banks, the unpurified bulk material, as well as the final product with particular attention for viral clearance processes (Darling, 2002). Hardly any guidance has been provided for the extent of detecting possible contaminants in case animal cell cultures are used for in vitro research or diagnostic activities or for purposes other than therapeutics or production of biopharmaceuticals. It is recognized that there is no single test suitable for detecting all possible contaminants. Therefore, the choice of detection technique depends on the contaminating pathogen and often a combination of methods is necessary to enhance detection in important samples such as master cell banks. Table 3 gives an overview of the use, detection capability and limitations of assays that might be used to detect and/or identify adventitious agents.

Work with cell cultures from human or primate origin should generally be performed under BSL2 conditions. Containment level 1 may be considered if all manipulations occur in a Type II biosafety cabinet and the

### Table 3

Use, detection capability, and limitations of assays that can be used to detect adventitious agents.

<table>
<thead>
<tr>
<th>Test</th>
<th>Detection capability</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct detection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· Electron microscopy studies</td>
<td>· Virus and virus-like particles</td>
<td>· Low sensitivity</td>
<td></td>
</tr>
<tr>
<td>· Sterility tests (a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Indirect detection</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>· Infectivity assays with sensitive or indicator cell lines (b)</td>
<td>· Capability of screening a broad range of viruses</td>
<td>· Detects only infectious viruses and those that result in CPE, HA and HAD ≠ not appropriate to detect retroviruses</td>
<td></td>
</tr>
<tr>
<td>· in vivo tests (c)</td>
<td>· Highly sensitive</td>
<td>· Expensive and time consuming</td>
<td></td>
</tr>
<tr>
<td><strong>Immunodetection methods</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>· Mouse and hamster antibody production test (MAP, RAP and HAP) (d)</td>
<td>· Contaminants of rodent cells.</td>
<td>· Use of living animals, time consuming, some viral antigens may not lead to antibody production</td>
<td></td>
</tr>
<tr>
<td>· Immunofluorescence and enzyme immunoassays.</td>
<td>· Non-cytopathic viruses (like BVDV).</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Molecular detection methods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>· PCR</td>
<td>· Broad range of pathogens.</td>
<td>· Specific and powerful test</td>
<td>· Cross-reaction of primer sequences with cell specific DNA; does not indicate whether virus is infectious</td>
</tr>
<tr>
<td>· Reverse transcriptase detection (e)</td>
<td>· Retroviruses</td>
<td>· Only detects enzymes with optimal activity under preferred conditions, interpretation may be difficult due to presence of cellular enzymes</td>
<td></td>
</tr>
</tbody>
</table>

(a) Samples are directly transferred on mycoplasma broth or rich broth and incubated at room temperature and at 37°C. Samples are then screened on signs of microbial growth.

(b) Detector cells are observed for cytopathic effects (CPE), hemadsorption (HA) or hemagglutination (HAD).

(c) inoculation of lysate of cells in live animals

(d) the lysate of cells and their culture medium are inoculated into SPF mice, rats or hamsters; the developing of antibody response reveals the presence of a virus

(e) based on activity test of the reverse transcriptase
cell culture is a well-characterized and certified cell line that presents no increased risk resulting from genetic modification or contaminating pathogen. However, as the implementation of good laboratory practices and the use of a BSC is usually the norm in most laboratories dealing with cell culturing, those laboratories can be upgraded to BSL 2 facilities by implementing a restricted number of simple additional safety measures. As biosafety

**Figure 1.**
Guidance for the assignment of the containment level.
measures aim at providing a maximal protection of human health and environment, it should be emphasized that horizontal laminar air flows and clean benches offer no protection for the manipulator or the environment, though it may guarantee protection of cell cultures from adventitious contamination. Therefore the use of a horizontal laminar "clean bench" should be prohibited.

Based on, but not limited to, key features of risk assessment and the type of manipulation performed as discussed in former paragraphs, Figure 1 presents a schematic guidance for the assignment of an appropriate containment level when manipulating cell cultures. This flowchart is only indicative and should be applied and/or reconsidered according to case specific conditions and risk assessments.

Conclusions

Bearing in mind the restricted survival capacity of animal cell cultures in non-optimized conditions of growth and recognizing that many cell lines have a long history of safe use, it may seem unlikely that these cells may cause harm to humans, animals or plants. Actually, the main hazard associated with the manipulation of cell cultures resides in the fact that they may harbor adventitious agents, which are often hard to detect and, hence, less controllable. In contrast to their host cells, adventitious agents may have the capacity to survive in more hostile conditions and may present risks for human health or the environment in case they are pathogenic. Consequently, a risk assessment of cell cultures will often lead to a risk assessment of the potential adventitious contaminants; the agents used for immortalization of the cells (viruses, viral sequences, etc.) and/or the agents that have been used for deliberate infection of these. Therefore, though the assignment of containment requirements cannot be generalized and should be performed on a case-by-case basis, it is recognized that most of the containment measures primarily aim at protecting cells from adventitious contamination in order to minimize potential risks for the manipulator.

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Biological Agents and Toxins Act: Development and Enforcement of Biosafety and Biosecurity in Singapore

Tin Tun, Kristen E. Sadler, and James P. Tam
Nanyang Technological University, School of Biological Sciences, Singapore

Abstract

Biosafety and Biosecurity are very important issues in the present climate where emerging infectious diseases and threats of bioterrorism and biological attacks are of global concern. Biological safety requires the strengthening of the regulatory requirements and operational and safety programs. Singapore’s first-ever law on the use of biological agents and toxins has come into force. The law clearly defines facility requirements for handling of them; develops the comprehensive system of control; prevents bioterrorism by controlling the use of high-risk hazardous agents; and, establishes a strong national biosafety culture. Singapore has made significant progress in the development and enforcement of biosafety and biosecurity measures. This progress certainly facilitates the safe expansion of our emerging biomedical industry and research development in life sciences. For the benefit of other facilities and future users, we report the impacts and contents of the new act in a simple and understandable presentation.

Introduction

The Biological Agents and Toxins Act (BATA) came into force on 3 January 2006 in Singapore (Ministry of Health, 7 December 2005). In writing the legislation, recommendations from the National Biosafety Committee (NBC) and its Technical Working Committee (TWC), which are represented by related government agencies, research institutions, hospitals and key industry players were taken into considerations. A public consultation exercise was conducted by the Ministry of Health (MOH) for the draft BATA from 11 April 2005 to 14 May 2005. Singapore’s first-ever law on the use of biological agents (BA) and toxins was approved by the Parliament on 18 October 2005 (The Straits Time, 19 October 2005).

On recommendation of the NBC the MOH has adopted the Laboratory Biosafety Manual, 3rd edition (World Health Organization, 2004) as the national guidelines for biosafety to supplement the BATA. The World Health Organization (WHO) has long recognized that biosafety and biosecurity are important international issues. The WHO manual encourages countries to prepare specific codes of practices for the safe handling of potentially hazardous agents and provides expert guidance for developing such codes of practices. The importance of personal responsibility for safe laboratory activities is stressed throughout the manual. A safe and healthful laboratory environment is the product of individuals who are trained and technically proficient in safe practices.

Previously, the MOH adopted guidelines regarding human pathogens, and these guidelines were used to determine the level of biosafety required for specific purposes. The guidelines included regulations regarding the import, transport, transfer, handling, and disposal of human pathogens and their risk group classifications (Disease Control Branch, 2004). Those regulations have been integrated into the sections of the present BATA.

Biological Agents and Toxins Act (BATA)

The BATA prohibits and otherwise regulates the possession, use, import, transshipment, transfer and transportation of biological agents, inactivated biological agents and toxins that are of public health concern (Singapore Statutes online, 2006). Its objectives include preventing acts of bioterrorism, establishing a strong national biosafety culture and facilitating emerging bioscience industry in Singapore. The important objectives of the BATA are provision of safety practices in handling of BA and toxins and promotion of biosafety training.

Major components of the BATA include lists of BA and toxins, controls for importation, possession, transshipment and transfer, and transport requirements. It also defines the facility requirements for high risk biological agents and toxins. The BATA adopts a schedule system for risk group classification of biological agents.

The BATA is relevant to companies and institutions involved in biomedical and life sciences research working with biological agents and toxins listed in schedules. Courier service providers should also be aware of the requirements for the transfer, transportation, transshipment and importation of biological agents and toxins. Any person who contravenes the sections of the Act shall be guilty of an offense and shall be liable on conviction to be pun-
ished with a very heavy fine or with imprisonment or with both.

**Schedules versus Risk Groups**

The risk group classification of hazardous agents does vary from country to country, even though there are global standard laboratory practices and many aspects of laboratory culture are shared throughout the world. An agent classified into Risk Group 2 in one country may be classified as Risk Group 3 in another. Dengue Virus Type 1-4 was classified as Risk Group 2 in Singapore (previously), Canada and Australia but classified as Risk Group 3 in Belgium and European Union (Tun et al., 2006). Under the present BATA, biological agents and toxins are classified into schedules.

The BATA differentiates between higher risk group and lower risk group BA, and also those with potential to be weaponized. Five schedules in BATA cover a wide spectrum of biological agents and toxins and different levels of controls have been adopted for each schedule. Table 1 shows an overview of the schedules with corresponding risk groups, descriptions of schedules, facility requirements and number of BA in each schedule (MOH Biosafety, 2006). Schedule 1 is separated into part I and part II based on their potential to be weaponized. Schedule 1 (part II) and schedule 2 biological agents are deemed to be of bioterrorism potential and facilities wishing to work with these BA must be officially recognized as a protected place under the Protected Areas and Protected Places Act.

**Table 1**

Five schedules in BATA with their descriptions, corresponding Risk Group, number of BA in each schedule and Facility Requirements.

<table>
<thead>
<tr>
<th>Schedule Classification</th>
<th>Risk Group</th>
<th>Descriptions of Schedule</th>
<th>No. of BA</th>
<th>Facility Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schedule 1 (Part I)</td>
<td>3</td>
<td>(1) Potential to cause serious disease which is high risk to individual</td>
<td>56</td>
<td>BSL3 Certified</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Uncertified facility can appeal)</td>
</tr>
<tr>
<td>Schedule 1 (Part II)</td>
<td>3</td>
<td>(1) Potential to cause serious disease which is high risk to individual</td>
<td>23</td>
<td>BSL3 Certified</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) Potential to be weaponized</td>
<td></td>
<td>and Protected Place</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Uncertified facility and protected place can appeal)</td>
</tr>
<tr>
<td>Schedule 2</td>
<td>4</td>
<td>(1) Can cause severe/lethal disease, high risk to individual and community</td>
<td>14</td>
<td>BSL3 Certified and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) Potential to be weaponized</td>
<td></td>
<td>Protected Place with</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Special Approval</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>granted by the Director (Medical Services)</td>
</tr>
<tr>
<td>Schedule 3</td>
<td>2</td>
<td>(1) Can infect humans</td>
<td>3</td>
<td>Specified in the Approval</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2) Need special attention in large scale production</td>
<td></td>
<td>by the Director (Medical Services)</td>
</tr>
<tr>
<td>Schedule 4</td>
<td>2</td>
<td>(1) Can infect humans</td>
<td>250+</td>
<td>Conditions of a Permit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>granted by the Director (Medical Services)</td>
</tr>
<tr>
<td>Schedule 5</td>
<td>–</td>
<td>(1) Microbial toxins with potential to be weaponized</td>
<td>5</td>
<td>Protected Place and</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Conditions of an Approval granted by the Director (Medical Services)</td>
</tr>
</tbody>
</table>
Biosafety Branch

The MOH established the Biosafety Branch in 2005 to account for all biosafety matters in a sustainable and organized manner. The branch administers the BATA; promotes high standards of biosafety in the research and biomedical community; maintains selected BA lists in schedules; assesses certification bodies and training providers; maintains a biosafety Information Technology (IT) system; coordinates containment measures and investigations; promotes links with other international and national biosafety organization; and, prevents bioterrorism by controlling the use of high risk biological agents.

According to the BATA requirements, a list of BA which is of importance to public health concerns and categorized in schedules based on their risk assessments, has been developed and updated. The branch keeps lists of Approved Facility Certifiers (AFC) and Approved Training Providers (ATP), and also sets the topics for biosafety training and the checklists for facility certification and for biosafety audit purpose. A webpage has been created and all relevant information is available online for the Biosafety Level 3 (BSL-3) facilities in Singapore (MOH Biosafety, 2006). The biosafety IT system allows all users to perform activities such as registering the facility, applications for permits and approvals, notification of transfers, receipt, inactivation and disposal, reporting of incident and inventory for biological agents, etc.

Possession Control

Approval to possess biological agents in schedules 1 and 2 and toxins in schedule 5 are agent-specific and granted by the Director of Medical Services. No approval to possess is required for BA in schedules 3 and 4. There is no expiration date for the approval; however, the validity of the approval is tied to the facility being a certified BSL-3 facility. The approvals to possess BA in schedule 1 (part II) and schedule 2 are granted to a certified BSL-3 facility which are also officially recognized as a protected place under the Protected Areas and Protected Places Act. The approval to possess toxins in schedule 5 is granted to a certified or uncertified facility which is officially recognized as a protected place.

A special approval to handle materials is applicable to schedule 2 BA and is only granted by the director under the circumstances that the use of the schedule 2 BA is necessary in public interest; and the person who requires the schedule 2 BA has put in place adequate measures to contain the risks to public health and security posed by the agent. The approval to possess a schedule 1 BA may be granted to an operator of uncertified facility if the director is satisfied that the activity involving the use of schedule 1 BA will be carried out at such facility in a safe and proper manner. Therefore, appeal for exemption to handle the BA under schedule 1 at an uncertified facility can be submitted to the MOH for approval from the Institutional Biosafety Committee (IBC) with risk assessment, documents to include research proposals and protocols, and proof that the facility is officially recognized as a protected place in case of schedule 1 (part II) biological agents.

To work with zoonotic agents, approval for possession of BAs from Agri-Food Veterinary Authority (AVA) of Singapore is required. The AVA inspects the facility before granting an approval. For genetically modified organisms (GMO), approval must be sought from Genetic Modification Advisory Committee (GMAC) before submitting an application for approval and permit to MOH.

In instances where a person possesses (and works with) a biological agent in contravention of sections in the BATA, the director may order the immediate cessation of any activity; the closure or cordoning off of the facility until such time as the director is satisfied that the facility may safely resume operation; the destruction of the BA at the facility; and, the decontamination of the facility. The person at the facility should undergo medical examination and medical treatment or may be quarantined at such place and for such period as specified in the order.

Import Control

All biological agents in the schedules require import permit. The applications for import permits can be submitted online at Singapore Customs Tradenet System (Singapore Customs, 2006). The importer of the BA into Singapore must have approval to possess the BA. The importer or the end-user is responsible for ensuring that a valid permit is obtained before bringing the BA into Singapore. Even when engaging a courier service provider to apply for the import permit on behalf of the institution, the importer must verify that the correct permit has been obtained from the MOH.

Transshipment of BAs under the BATA must be carried out in accordance with the conditions of a permit granted by the director. Transshipment refers to instances where Singapore acts as a port of transfer for BAs and where the BAs arrive and are stored temporarily at any given location in Singapore. This is regardless of whether the BAs remain on the original conveyance (i.e., plane) upon which they arrive in country or are transferred to another conveyance (i.e., another flight). Every permit to import or transship a biological agent is valid only for the one specific consignment of the BA for which the permit has been granted, and can not be used for other consignments. Under the BATA the importer has to notify the MOH of any failure to receive schedules 1 (part II) and schedule 2 biological agents and schedule 5 toxins within 24 hours of such time as reasonably estimated for the receipt.

When temporary storage is required at any location
for imported BA before it is delivered to the destined facility in Singapore or for transshipment from Singapore, the permit holder must ensure the storage of the BA is in accordance with requirements as prescribed by the BATA.

For importation of zoonotic agents, the importer is required to obtain import permits from both the AVA and the MOH. The AVA and MOH have streamlined the import permit processing on Singapore Customs Tradenet System. Importer is required to declare a set of MOH-AVA product code for each zoonotic agent (AVA, 2005).

The BATA does not have requirements for export of biological agents and toxins. Export of high-risk biological agents and toxins is controlled by the Strategic Goods Control Act administered by Singapore Customs.

### Transfer Control and Transport Control

For transfer of schedule 1 and schedule 2 BA or schedule 5 toxins between facilities within Singapore, the BATA requires that both transferor and transferee must have valid approval to possess that agent. The transferor is responsible to notify the MOH of the proposed transfer. Transferor needs to notify the transferee of an estimated time of receipt of the agent and provide the carrier of the agent with a 24-hour emergency contact number of a person who is responsible for the transfer process. In the event of failure to receive the agent, the transferee must notify the MOH. Table 2 shows the approvals, permits and requirements to handle the BA under the BATA.

Packaging of the BA uses a basic triple packaging system which comprises a primary receptacle, secondary packaging and an outer packaging. Biological agents are packaged to meet International Air Transport Associations (IATA) regulations.

### Facility Requirements

BSL-3 facility must be certified by a MOH-approved facility certifier (AFC) before applying for approval to possess schedule 1 and schedule 2 biological agents. The facility needs re-certification yearly or upon any design or structural change made to the facility. The MOH Biosafety keeps a checklist for certification process which is derived from the WHO Laboratory Biosafety Manual third edition and additional items recommended by the NBC as well as legislative requirements by the BATA. The facility that wants to handle schedule 1 (part II), schedule 2 biological agents and schedule 5 toxins, must be declared as a protected place under the Protected Areas and Protected Places. Additional criteria may be required by the AVA for Animal BSL-3 facility.

It is a requirement for a BSL-3 facility to establish an Institutional Biosafety Committee (IBC) which includes at least biosafety coordinator, microbiologist, facility maintenance personnel and representative from senior

### Table 2

Summary of approvals and permits required to handle biological agents under the BATA.

<table>
<thead>
<tr>
<th>Approval/Permit Requirement</th>
<th>Schedule of BA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1(I)</td>
</tr>
<tr>
<td>Approval to possess</td>
<td>Yes</td>
</tr>
<tr>
<td>Approval to produce (Large scale)</td>
<td>Yes</td>
</tr>
<tr>
<td>Special Approval to handle</td>
<td>NA</td>
</tr>
<tr>
<td>Import permit</td>
<td>Yes</td>
</tr>
<tr>
<td>Notify Failure of Receipt of Import</td>
<td>No</td>
</tr>
<tr>
<td>Transshipment permit</td>
<td>Yes</td>
</tr>
<tr>
<td>Transfer Notification in Singapore</td>
<td>Yes</td>
</tr>
<tr>
<td>Notify Failure of Receipt of Transfer</td>
<td>Yes</td>
</tr>
<tr>
<td>Certified BSL3 Facility</td>
<td>Yes*</td>
</tr>
<tr>
<td>Protected Place</td>
<td>No</td>
</tr>
</tbody>
</table>

NA = Not applicable
* = Uncertified facility may appeal for exemption
management. The IBC is responsible to formulate and review biosafety policies and programs including training of staff, conducting risk assessment and approving the research projects. All activities involving schedule 1, schedule 2 and schedule 3 (large-scale production) biological agents and schedule 5 toxins must be endorsed by the IBC before submitting the applications for approvals and permits or reporting to the MOH.

Every facility appoints a biosafety coordinator who participates in a recognized biosafety course conducted by the MOH-approved training provider (ATP) and passes the competency test administered by the MOH. Training for BSL-3 staff is mandated under the BATA. Such training for personnel working in a BSL-3 facility can be conducted in-house or by experienced external trainers. The BATA has given a six-month transitional period from the date of commencement of the BATA to BSL-3 facilities in Singapore for necessary arrangements and preparations to meet the BATA requirements.

**Activities Exempted from the BATA**

The act will not apply in relation to the use of biological agents and toxins for certain purposes which include disposing of any biological agent by a hazardous waste contractor; the handling of any agent in the course of carrying out a diagnosis and an autopsy; and collecting of food samples or samples from the environment for the purpose of carrying out laboratory analysis to determine or identify for public health purpose the nature of any biological agent that is present in such samples. Exemptions are also given to the use or possession by any person lawfully manufacturing, supplying, selling or dispensing the finished cosmetic or medicinal product; any registered practitioner using the finished cosmetic or medicinal product in the course of treating another person; and, any person using those product for cosmetic or medical purpose for which it is intended.

**Conclusions**

The regional and global spread of emerging infectious diseases has driven Singapore to put emphasis on research in life sciences and to develop comprehensive system of control for potentially hazardous biological agents. The strict rules have been introduced to protect people from being exposed to the potentially hazardous biological agents and also to prevent terrorists from turning research samples into biological weapons. The laboratory operating environment needs to be defined in terms of biosafety and biosecurity capabilities. Where threats of bioterrorism and biological attacks are of global concern, the measures used to secure a facility storing high-risk agents are of the utmost importance. If biological agents and toxins fall into the wrong hands, the destruction and dangers posed will be indescribable. A good regulatory framework will strengthen Singapore’s standing as a biomedical hub and help it to attract world-class researchers. The laws have to strike a good balance between keeping research safe and being too restrictive. The requirements should not result in unnecessary cost increases, research being hampered, and scientists being discouraged from working to prevent disease outbreaks. There is a fine of up to $1 million Singapore dollars and life imprisonment if charges involve a deliberate attempt to use biological agents and toxins for biological warfare or any non-peaceful purpose. Such severe penalties for convicted offenders reflect Singapore’s serious commitment to biosafety and biosecurity.

**References**


Book Review

Reviewed by Leslie Delpin

University of Connecticut, Storrs, Connecticut

The Power of Plagues

By Irwin W. Sherman
431 pp., illustrated

In the preface to The Power of Plagues, the author lays out the book’s three objectives: 1) “to place infectious disease in a historical context...”; 2) “to describe the nature and evolution of diseases...”; and 3) “to show how the past can prepare us for future epidemics with infectious diseases.”

The author does a nice job of presenting the historical, societal and cultural aspects of key epidemic diseases (six plagues of antiquity, bubonic plague, AIDS, cholera, typhus, malaria, smallpox, syphilis, tuberculosis, leprosy, six plagues of Africa and emerging plagues) and devotes one of the book’s 17 chapters (Chapter 16) to noninfectious plagues (pellagra, beriberi, scurvy and rickets) that resulted from dietary (micronutrient) deficiencies.

In Chapter 2 (Plagues, the Price of Being Sedentary), the author describes how a sedentary lifestyle contributed to disease spread and plague when hunter-gatherers settled down and became farmers. Chapter 11 (The Plague Protectors) provides an interesting overview of first uses of antiseptics, anesthesia and antimicrobial drugs.

The author disappoints in his objective to describe the nature and evolution of diseases. The description of the causes of disease outbreak and the dynamic and multifactor interaction between host, disease agent and environment are one-dimensional and undeveloped. The author also disappoints in his objective to show how the past can prepare us for future epidemics. He provides very limited description of successful disease eradication campaigns of the past.

In the preface, the author states that the text is intended to be accessible to readers with no scientific background. While some comprehensive concepts are explained effectively using creative analogies, there is considerable scientific and medical language used throughout the text. The inclusion of a glossary of terms would have benefited the non-scientific reader.

Although there are some factual inaccuracies related to antivirals, BCG vaccination and historical material, The Power of Plagues is, for the most part, very readable and enjoyable. Individuals with an interest in the history of epidemic diseases or who have an interest in history (with a little smattering of anthropology thrown in) will find this text entertaining reading. The reader will also become familiar with terms such as “high-tech cannibalism” and “fatal brain plague.”

WHO Releases Action Plan to Increase Pandemic Influenza Vaccine Supply and Guide for Collecting, Preserving and Shipping Specimens for Diagnosis of H5N1

At the end of September, the World Health Organization (WHO) issued a new report, “Global pandemic influenza action plan to increase vaccine supply.” The action plan was developed by a consensus of the world’s experts in influenza, immunization, vaccine research, and manufacturing. The report can be downloaded by going to: www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_2006_1/en/index.html

The WHO also released a guide for field operations called, “Collecting, preserving and shipping specimens for the diagnosis of avian influenza A (H5N1) virus infection.” The Guide can be downloaded at: www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1/en/index.html
Do you have a biosafety question and you’re not sure who to ask? Send your questions to the “Ask the Experts” column and I’ll get them answered for you. Drawing from my own experience or that of other experts in the field, we’ll try to compile a thorough and comprehensive answer to your question. Please e-mail your questions to jkeene@biohaztec.com or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

**Biosafety Cabinets and Medical Waste**

**Question**

We have three biological safety cabinets. All are class II, one is type A; the other two type B. All three passed their annual certification. In our laboratory, we use *Standard Methods 21st Edition* as a reference for monthly QC Equipment Checks. In 9020B, part m it states: “Once per month, expose plate count agar plates to air flow for 1 hr. Incubate plates at 35 degrees C for 24 hours and examine for contamination. A properly operating safety cabinet should produce no growth on the plates.” Instead of one plate, we place 3 plates in the cabinet, one at each end and one in the middle. The last 3 months, we have seen one colony on different plates in different cabinets. We have repeated the test several times with the same results. The same sample plate does not grow a colony every time it is exposed. Finally, this month all plates have shown no growth. Could you provide some guidance on interpreting these results?

**Response**

Biosafety cabinet performance analysis is done in accordance with the National Sanitation Foundation (NSF) Standard 49. In this standard, there is a requirement that all biosafety cabinets pass a Product Protection Test. The test involves generating an aerosol containing \(10^8\)/ml spores positioned at the level of the work surface, in the center of the cabinet, and 4 inches outside of the work opening. The work surface is covered with open plates and the test is run for 5 minutes, after which the plates are covered and incubated. Under these very harsh conditions, a test is considered acceptable if the number of CFU’s within the cabinet does not exceed 5 CFU per test.

While “Standard Methods” states that “a properly operating safety cabinet should produce no growth on the plates,” the universally accepted standard for biosafety cabinetry is the NSF 49 standard. This standard requires that under the test conditions, which should be done by certified testing personnel, the acceptable level must be less than 5 CFUs per test. Having said this, placing a single plate inside a cabinet and leaving it there for an hour should show no growth. However, because you are looking at random events, the test could show a positive result and the cabinet could still be functioning properly. You have placed three plates in the cabinet and that would increase the probability of capturing the random particles that might enter the cabinet, thus giving you a positive result.

A number of factors could affect the results of your testing. These would include personnel movement within the laboratory, operation of equipment that could affect airflow in front of the cabinet during the test, fluctuation in the HVAC system in the laboratory, personnel entering or exiting the laboratory at the time of the test and others. Because your “failures” are not consistent and not associated with either a particular cabinet or location within a cabinet, it does not seem probable that the cabinets are at fault, but rather random circumstances within the laboratory may be the problem.

Generally, if a BSC is properly certified on an annual basis, by a certified certifier, then the cabinet is working properly and any contamination occurring inside the cabinet is most likely due to a failure of the lab personnel to work properly within the cabinet. While doing lab safety and biosafety training, I have consistently found that only about 10% of the people who have BSCs in their lab actually know how the cabinets work. This lack of knowledge continues in spite of many excellent sources of information on BSCs and the continued effort of Biosafety Professionals to teach good practice.

**Question**

Our Laboratory SOP on Biohazards defines a biohazardous waste as follows: "a waste is considered to be infectious, and thus a biohazard, when it contains pathogens..."
of sufficient virulence, and in sufficient quantities that an infection may occur when a susceptible host is exposed to the waste.” Because of this definition, all samples from hospitals, medical laboratories, clinics and offices, diagnostic laboratories, animal experimentation units, research laboratories and dental offices are classified as biohazards and must be handled per the SOP using proper PPE. Years ago it was “determined” that any sample from the above locations that had a pH of <2 or >12 would not be considered a biohazard because of the extremes of pH. I was told that EPA, OSHA or CDC had provided the information. I have not been able to find any documentation.

Response

EPA defines medical waste as follows: “Medical waste is generally defined under state regulations. Medical waste is often described as any solid waste that is generated in the diagnosis, treatment, or immunization of human beings or animals, in research pertaining thereto, or in the production or testing of biologicals, including but not limited to:

- blood-soaked bandages
- culture dishes and other glassware
- discarded surgical gloves—after surgery
- discarded surgical instruments—scalpels
- needles—used to give shots or draw blood
- cultures, stocks, swabs used to inoculate cultures
- removed body organs—tonsils, appendices, limbs, etc.
- lancets—the little blades the doctor pricks your finger with to get a drop of blood.”

In addition, EPA gathered information during the two-year period of the Medical Waste Tracking Act and concluded that, “the disease-causing potential of medical waste is greatest at the point of generation and naturally tapers off after that point. Thus, risk to the general public of disease caused by exposure to medical waste is likely to be much lower than risk for the occupationally exposed individual.”

Currently, Medical Waste handling, transportation, and disposal are not regulated by the USEPA, but may be regulated on a “state-by-state” basis. Each facility should first determine whether or not there is a state regulation that applies and then follow that regulation.

If you are using the definition stated above and are considering all materials from those locations as biohazardous, then you are probably going too far. Note the definition requires two things: 1) the presence of pathogens of sufficient virulence, and 2) sufficient numbers of those pathogens. These criteria are not always met by all specimens or materials collected in the noted locations. The determination of which wastes meet the definition requires a realistic evaluation by the generator. Laboratory personnel should always evaluate the materials with which they are working and, using reasonable criteria, differentiate between what is regulated and what is not.

You do not state what your proscribed PPE is for work with the medical waste, but again I would suggest that you evaluate the situation and your procedures in order to allow for reasonable interpretation and appropriate protection without undue burden on your personnel. Not all specimens from the above-mentioned locations are biohazardous and those that may contain infectious agents in small numbers, while potentially hazardous, can be handled differently than the cultures from those same specimens, which by virtue of their growth, would contain high numbers of organisms. As stated above by the EPA, the potential risk of exposure to infectious agents in Medical/Regulated Waste is far greater at the point of generation than once it has left the facility.

With regard to the pH issue, I can find no reference in either the CDC, EPA or OSHA documents on Regulated Waste that specifically state that materials potentially containing pathogens and had a pH of <2 or >12 would not be considered a biohazard because of the extremes of pH. (If someone reading this has found such documentation, please share it with us all.) Many factors could contribute to the survival of pathogens at either extreme acid or basic conditions. One thing we can say is that it is unlikely most human or animal pathogens would be viable in materials at either a pH <2 or pH >12. However, it is possible for them to be present in such materials. We do know that some intestinal organisms, under specific conditions, can survive at very low pHs. Therefore, if you are asked to look for infectious agents in materials at extreme pH ranges and someone thinks they might be present, the material should be treated as potentially biohazardous until it is shown not to be biohazardous.

References


U.S. Environmental Protection Agency web site at www.epa.gov
Biosafety Tips
Karen B. Byers
Dana Farber Cancer Institute, Boston, Massachusetts

Biosafety Tips brings you practical approaches to biosafety or “news you can use.” If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of workplace situations. I welcome feedback or suggestions for future topics. Please e-mail any comments or suggestions to karen_byers@dfci.harvard.edu or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net.

Neisseria meningitidis Exposures in Clinical Laboratories

In the United States, approximately 3,000 times each year, clinical microbiology laboratories isolate Neisseria meningitidis and another diagnosis of meningococcal meningitis is confirmed. The reported fatality rate for community-acquired meningococcal disease due to N. meningitidis is 10-14%; 11-19% of those who recover have permanent hearing loss, mental retardation, loss of limbs, or other serious sequelae (Bilukha, 2005). The fatality rate of laboratory-acquired infections (LAIs) with N. meningitidis is estimated to be as high as 50% (Sejvar, 2005). To prevent this disease, the Advisory Committee for Immunization Practices (ACIP) recommends the tetravalent meningococcal polysaccharide-protein conjugate vaccine for the following risk groups: “young adolescents, college freshmen living in dormitories and other populations at increased risk (i.e., military recruits, travelers to areas in which meningococcal disease is hyperendemic or epidemic, microbiologists who are routinely exposed to isolates of Neisseria meningitidis, patients with anatomic or functional asplenia, patients with terminal complement deficiency, and persons exposed to active or passive tobacco smoke)” (Bilukha, 2005). The vaccine reduces the risk of infection from serogroups A, C, Y, and W-135. Unfortunately, it does not protect against serotype B. The importance of various serotypes in causing clinical infection varies according to geographic regions. In sub-Saharan Africa, there is a “meningitis belt” with large seasonal epidemics caused by serotype A, C, and W-135. In Asia, most infections are caused by serotype A; New Zealand reports a large number of cases from serotype B. The majority of the cases in the Americas and Europe are caused by both serotypes B and C (World Health Organization, 2003).

To prevent occupational infections from N. meningitidis, CDC recommends the use of the biosafety cabinet, an important Biosafety Level 2 engineering control, for handling isolates from sterile sites such as blood, cerebrospinal fluid, or inner ear fluid. The bacteria can be found in the pharyngeal exudates of some healthy carriers, but pharyngeal isolates are generally considered less invasive and have not caused any reported laboratory-acquired infections (Sejvar, 2005). Occupational infections are linked to manipulation of the invasive isolates of N. meningitidis from sterile sites such as blood, cerebrospinal fluid, or inner ear fluid. Clinical microbiologists may find it inconvenient to use a biological safety cabinet to manipulate blood and cerebrospinal fluid cultures, since these procedures were previously carried out on the open bench, but consistent use of the biosafety cabinet is critical to prevent rare, but serious, cases of occupational transmission. For example, one fatal laboratory-acquired infection occurred from the first N. meningitidis isolation, which had occurred in that laboratory in four years (CDC, 2002). Experienced microbiologists who routinely handle N. meningitidis have also been infected. In one case, an experienced scientist worked with suspension cultures on the open bench without incident. After this individual was hospitalized, a spinal fluid isolate was determined by restriction fragment length polymorphism to be identical to one of the three Brazilian strains studied in the lab the previous week (Knudsen, 1992). A fatal infection also occurred in a state laboratory, the infected microbiologist handled an average of four N. meningitidis isolates a month (CDC, 2002).

In a recent review of laboratory-associated infections, Harding and Byers (2006) cited 31 cases of N. meningitidis infections in clinical microbiologists that had occurred between 1979-2004; 11 were fatal. Five cases were described in a retrospective study in England and Wales during the 15-year period between 1 January 1985 and 31 December 1999 (Boutet, 2001). Boutet reported that all of the infected microbiologists had prepared suspensions on the bench, outside of biosafety cabinet, within seven days before the onset of meningococcal disease. The review data available on laboratory-acquired infections with N. meningitidis can be summarized with the following state-
ment. In 30 out of 31 cases, the infected microbiologist had handled \textit{N. meningitidis} isolates on the open bench, usually within 3-4 days of becoming ill, and the isolates from the original patient and the laboratory-acquired case were identical in serotype and other markers.

**Procedures Associated with LAIs**

Identification procedures for \textit{N. meningitidis} begin with “preparing a heavy suspension.” Specific procedures performed by the clinical microbiologists who became infected were:

- Slide agglutination testing and observing colony morphology (CDC, 2002);
- Aspirating samples from blood culture bottles, performing Gram stains, handling plates, and subculturing cerebrospinal fluid (CDC, 2002);
- Culturing blood and subculturing isolates (CDC, 1991);
- Performing agar diffusion antibiogram with serogroup C strain isolated from cerebrospinal fluid (Guibordenche, 1994);
- Determining antibiotic susceptibility using a Steers-type replicating device with 10 isolates (Guibordenche, 1994);
- Preparing a suspension, vortexing, seeding agar plates, and pipetting the suspension into the wells of a plate to perform a commercial identification procedure on the workbench (Paradis, 1994);
- Making a heavy suspension of \textit{N. meningitidis} to inoculate an identification test strip using a pipette, by collecting a colony with a swab, and suspending it in saline (CDR, 1992); and
- Preparing negative stains for electron microscopy by adding 0.1 ml bacterial suspension to 0.9 ml 3.5% formalin solution (Bhatti, 1982).

In 2002, CDC reported two fatalities from meningococcal laboratory-acquired infections in Mortality and Morbidity Weekly Report and they requested information on additional case reports in the article, as well as on websites and email lists of various infection control and infectious disease organizations. Responders provided case reports from the United States, Canada, Germany, Switzerland, and Australia that had occurred between 1985 and 2000. Dr. Sejvar kindly compared the CDC case reports with the literature reports, eliminated overlapping reports, and confirmed that, in the period between 1979 and 2004, information was available on 31 laboratory-acquired infections with \textit{N. meningitidis} (Sejvar, personal communication). In 2006, Sejvar et al. published an analysis of the occupational case reports of \textit{N. meningitidis} infection that occurred between 1985 and 1999. Sixteen infected microbiologists performed the following procedures with \textit{N. meningitidis}: examining Petri solid medium plates (50%), subculturing isolates (50%), and performing serogroup identification (38%). Fifty-six percent of the 16 laboratory-acquired infections were due to serogroup B and 44% of the infections were due to serogroup C. Fifty percent of the cases were fatal (Sejvar, 2005).

After the analysis was completed, CDC received three additional reports of laboratory-acquired infections in 2002. All three staff members had worked with \textit{N. meningitidis} on the open bench; two of the staff members had worked behind a standard splash shield (Sejvar, 2005). All of the occupational infections were acquired in the microbiology laboratory; the infections occurred after working with \textit{N. meningitidis} cultures on the open bench, not from handling body fluids from meningitis patients.

**Route of Transmission**

\textit{N. meningitidis} is transmitted from person to person by droplets of respiratory or throat secretions; close and prolonged contact is required for community-acquired infections (World Health Organization, 2003). In the laboratory setting, \textit{N. meningitidis} is transmitted by aerosol or droplets. In the 31 cases reported, only the individual actually subculturing the isolate, preparing the suspension, or setting up antibiotic susceptibility tests on the open bench were infected. Sejvar points out that some of the routine procedures conducted by the infected workers have not traditionally been considered high-risk for the generation of aerosols, e.g., transferring culture with a loop. Two of the infected workers used a splash shield (CDC, 2005); apparently, a splash shield is not sufficiently protective. CDC points out that the use of face shields or respirators cannot be evaluated from the information provided, and emphasizes use of the biosafety cabinet for manipulation of isolates from sterile sites.

**Host Factors**

Additional host risk factors are suspected in only two of the 31 LAIs with \textit{N. meningitidis} and both cases were fatal. One infected microbiologist had an upper respiratory infection at the time of exposure; this may have contributed to susceptibility (CDC, 1991) or possibly lead to contaminated hands touching mucous membranes. Another microbiologist was 1.5 meters tall and worked standing up at the bench. It was suggested that short stature increased the risk of work on the bench since the manipulations were closer to the breathing zone of the infected individual (Paradis, 1994). In the 31 case reports, the only documented lapse from established laboratory procedure occurred during the preparation of a laboratory strain of \textit{N. meningitidis} for electron microscopy. A technician did not wear gloves to prepare negative stains, which requires adding 0.1 ml of a suspension of \textit{N. meningitidis} to 0.9 ml of a formalin solution (Bhatti, 1982).
Incident Reporting and Prevention

There is only one anecdotal report of a “mishap.” The husband of one microbiologist told the ambulance crew that his wife had a mishap in the laboratory with a bacterial culture from a meningitis patient. No additional information is available. The microbiologist died and the incident was not reported to the laboratory director or coworkers (CDC, 1991). This reinforces that parenteral or mucosal exposures to N. meningitidis require prompt reporting and treatment. Microbiologists who find, after their cultures are identified, that they have inadvertently manipulated invasive N. meningitidis isolates in a manner that could induce aerosolization or droplet formation (including plating, subculturing, and serogrouping) on an open bench top should also consider antimicrobial chemoprophylaxis (CDC, 2002). In order to help prevent N. meningitidis infections, training for clinical microbiologists should emphasize use of the biosafety cabinet and personal protective equipment, provide information on the benefits and limitations of vaccination, and underscore that incidents must be reported promptly. Current information to guide the occupational health program in dealing with exposure incidents, and counseling on vaccination is available on the web at www.cdc.gov/mmwr/preview/mmwrhtml/rr5407a1.htm

References


Centers for Disease Control and Prevention, Division of Bacterial and Mycotic Disease. (2006). Meningococcal Disease. Available at www.cdc.gov/ncidod/dbmd/diseaseinfo/ meningococcal_t.htm


Fund Donations

ABSA thanks the many of you have generously contributed to the Richard C. Knudsen Memorial Fund. The proceeds from this fund will be used to establish an award to honor Rich’s memory. Those wishing to make donations to this fund should make their checks payable to the American Biological Safety Association. Please add a notation to the memo line that the check is to be used for the Richard C. Knudsen Memorial Fund. Checks should be mailed to ABSA, 1200 Allanson Road, Mundelein, Illinois 60060-3808.
Capsule

Ed Krisiunas

WNWN International, Burlington, Connecticut

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

OSHA News Release


The U.S. Department of Labor’s Occupational Safety and Health Administration (OSHA) has unveiled new safety and health guidance that alerts employees and employers about the hazards of occupational exposure to avian influenza from infected birds, or “avian flu,” and provides practical recommendations on ways to avoid infection.

OSHA Guidance Update on Protecting Employees from Avian Flu Viruses, as well as other important resource information on the topic, is available in English and Spanish by visiting the “In Focus” section on the home page of OSHA’s web site at www.osha.gov.


Recommended Adult Immunization Schedule—United States, October 2006—September 2007

The Advisory Committee on Immunization Practices (ACIP) annually reviews the recommended Adult Immunization Schedule to ensure that the schedule reflects current recommendations for the licensed vaccines. In June 2006, ACIP approved the Adult Immunization Schedule for October 2006—September 2007. This schedule has also been approved by the American Academy of Family Physicians and the American College of Obstetricians and Gynecologists.

Recommended Adult Immunization Schedule—United States, October 2006—September 2007

National Personal Protective Technology Laboratory (NPPTL)

A good source of information on respiratory protective equipment is the National Personal Protective Technology Laboratory (NPPTL). In 1999, at the request of Congress, the National Institute for Occupational Safety and Health (NIOSH) established the NPPTL as one of two major research laboratories in Pittsburgh, Pennsylvania.

NPPTL focuses expertise from many scientific disciplines to advance federal research on respirators and other personal protective technologies for workers. NPPTL’s efforts are essential for applying state-of-the-art science to meet the increasingly complex occupational safety and health challenges of the 21st Century. Information can be found at the following link:

www.cdc.gov/niosh/npptl/default.html

Wildlife, Exotic Pets, and Emerging Zoonoses (In “Emerging Infectious Diseases—January 2007”)

Many factors play a roll in the emergence or reemergence of zoonotic diseases. Chomel and colleagues review these issues in the January 2007 issue of Emerging Infectious Diseases.

Lovelace Respiratory Research Institute (LRRI), a private biomedical research organization in Albuquerque, NM, recently opened an advanced 9,500-sf ABSL-3 aerobiology laboratory after an intensive commissioning and validation process. The facility is used to study aerosolized pathogens on the CDC’s Select Agent List and for vaccine efficacy testing. Built on a strict 17-month timeline, the facility is subject to exacting regulatory requirements. To ensure that the facility would operate as expected and meet all safety requirements, the organization applied an intensive inspection and testing process dubbed, “CVQx” (commissioning, validation, and qualification).

“We in the biocontainment industry have a responsibility to our communities, co-workers, and employees to develop facilities that are safe and reliable over the long term. Engaging the CVQx plan helped toward achieving this objective and to assure that the lab was built on time and on budget to the highest possible standards,” says John Lopez, facilities director for LRRI.

LRRI is the only private organization in the country exclusively dedicated to the study of respiratory disease. The organization’s ABSL-3 inhalation research facility, which opened in 2005, will study exposure to a variety of airborne pathogens and toxins, including those potentially used in biological terrorism. Due to the nature of the research, the facility must have solid and liquid waste decontamination, HEPA filtration of all incoming and outgoing air, and completely redundant electrical, HVAC, and monitoring systems.

The facility uses an array of sophisticated equipment to simulate natural inhalation exposure. The majority of the building is BSL-3 rated and, since pathogens are aerosolized, personnel must wear individual breathing apparatus. Because the facility does work involving multiple select agents and animal species, and vaccine efficacy testing, it is subject to rigorous regulatory standards including CDC, USDA and FDA. These regulations stipulate that the performance of core facility elements be thoroughly inspected and tested prior to operation, and that all test results be documented.

To achieve this, a core team of project stakeholders—including representatives from the general contractor, architecture-engineer, vendors, subcontractors, and LRRI facility staff—was forged to execute the CVQx plan. Team members were carefully selected early in the planning process.

“The CVQx team needs to be as small as possible, and have someone with the authority to make final decisions, or things will get bogged down and the project won’t be delivered on time,” says Lopez.

Terms to Build On

Commissioning is the process of ensuring that a building functions according to design specifications. It is an essential part of containment facility construction. Commissioning actions—many of which are prescribed by government regulations and industry standards—can be handled by in-house staff, the architecture firm responsible for design, or a third-party expert.

The term validation, in the CVQx model, refers to validating the functionality of systems like computerized inventory, access tracking, and how digital records are secured.

“There are very specific procedures for managing electronic data in an FDA-regulated facility. If your records are all computerized, it adds a whole level of validation requirements. You have to be able to prove that the data are accurate, complete, and have not been modified. Security is paramount in that particular area,” says Lopez.

Qualification means confirming that the basic operational elements and subcontractors are qualified to meet the regulatory demands of the facility. This includes things like caging, network, and communications systems.

Engaging the Process

Identifying who will conduct the specific inspections and tests is an important step in CVQx implementation. Potential external resources include specialized system technicians, vendor subcontractors, and industry consultants. In-house resources include information services, facility operations staff, researchers, and quality assurance personnel. The optimum situation is a blend of both internal and external resources, but Lopez points out that finding qualified vendors can be a challenge.
“Thoroughly documented inspection and testing are not necessarily strong capabilities of many contractors. If external resources are used, careful evaluation of their commissioning experience is important,” says Lopez.

In order to maintain a high level of control over significant project details, the team handled facility commissioning internally, with the selective addition of specialized external support. The decision to perform most CVQx procedures in-house was driven by a number of factors including lack of qualified vendors and contractors, the greater flexibility in coordinating testing with internal personnel, the desire to maximize the value of the inspection and testing activities to train operating and maintenance personnel, and the necessity to perform ongoing commissioning and recommissioning.

LRRI staff wrote and executed all test scripts. In situations where external resources were used, internal personnel were assigned to provide oversight and gain procedural knowledge that contributed to the sustainability of ongoing operations. There were several areas where the team deemed it crucial to have in-house personnel actively involved in the testing, including HVAC controls, effluent decontamination, and sterilizers. This substantially improved knowledge retention and made for a smoother transition from construction to operation.

“We considered it mandatory for our most knowledgeable instrumentation and controls technician to be involved in the inspection and testing of the HVAC control system. The contractor can achieve basic functionality, but higher levels of performance are best achieved by the person responsible for ongoing operations,” Lopez says.

Risk Assessment

Conducting a thorough commissioning and validation process can be an expensive, time consuming operation. To control costs and prevent pre-commission occupancy, the team applied the principles of risk assessment in determining which issues to address.

“A functional CVQx plan must be based on sound risk assessment principles. Otherwise, cost and schedule can get out of hand. You have to look at each project element and activity and decide if it’s warranted relative to the effort, and risk,” Lopez says.

The CVQx plan constituted 7 percent of total project cost, with more than 800 components tested at a rate of approximately $650 a component.

“Obviously, when you go through this kind of process at this level, especially in terms of FDA compliance, it raises the bar considerably,” Lopez says.

Defining Scope

The team faced a wide range of considerations when choosing when and where to apply the CVQx procedure. It was important to decide early in the process what elements were going to be analyzed so the team could determine the best sequence and methodology for testing them.

“The fundamental performance objective was containment, environmental control, sterilization and decontamination, security, communications and alarm response and notification, so these established our initial scope. We focused on meeting CDC, USDA and FDA requirements because there were a lot of things we could do in this regard early in the process,” says Lopez.

The CVQx team used a pyramid-based model to identify the potential actions that might be executed for each component and system of the project. At the base of the model are fundamental procedures like pressure tests and physical material and installation inspections. Progressively moving up, the team looks at things like vendor qualification, factory inspections, performance tests, delivery inspections, and equipment calibration.

“Calibration can be a problem for contractors and it’s an important issue because if the test equipment and system components, such as differential pressure sensors, aren’t calibrated properly, then everything that happens afterward is suspect,” he says.

At the top of the CVQx pyramid is the functional testing of integrated operational elements like HVAC balance, biosafety cabinets, and decontamination systems. These systems were tested as early in the process as possible to identify and correct problems in anticipation of subsequent testing. In one case, the team tested the facility’s effluent decontamination system by pumping wastewater from another animal and laboratory facility through it.

“We could have waited to test that until we had waste coming from the building, but if there was a problem, it would have been a really big problem. So we tested the system by pumping waste through it from a comparable non-contaminated source. By the time we went operational, we had already run 160 batches through,” Lopez says.

Testing and Adjusting

Functional tests are intended to ensure that design requirements are met and to demonstrate the real life performance of systems and equipment once the building is operational. Functional tests were conducted to test integrated performance looking at the impact one system has on another, such as the impact of different HVAC systems; to test performance during abnormal operating conditions such as loss of facility power; to simulate operating conditions during normal operating and maintenance such as isolating rooms for decontamination; and lastly, to determine the maximum capacity of systems such as the effluent decontamination system. LRRI devel-
oped most of the forms and checklists used in the testing process including instructions for documenting results, failures, and corrective actions.

“Functional testing is really the ultimate performance measure. Until you test all the integrated systems and resolve any deficiencies, you don’t have an acceptable facility,” Lopez says.

More than 250 inspections and tests were conducted as part of the CVQx process. This resulted in the identification of 113 deficiencies that needed to be resolved. Some of these were the result of improperly executed tests, some due to contractor installation errors, and some were design issues. When a deficiency was discovered, it was resolved and then re-tested with detailed documentation of every step logged in a database.

“Resolving deficiencies creates great training opportunities. We learned something from every one of those deficiencies,” says Lopez.

Deficiencies were identified with the building automation system and with backup battery systems during simulated power outages; there were conflicts with doors and hardware as they related to directional airflow; and there were conflicts between systems identified during integrated functional testing. Each of these issues were identified in the CVQx process, resolved, and re-tested prior to facility occupancy.

“It is difficult to predict all of the interrelations from a design perspective and foresee the issues that come up when systems react in the real world. This is why a thorough commissioning and validation plan is essential for the safe operation of BSL facilities,” he says.

Biography

John Lopez is the major projects manager for the Lovelace Respiratory Research Institute in Albuquerque, N.M. He has been designing, building, and managing research facilities for more than 30 years. Lopez has been trained in the design, operation, and safety principles of biocontainment facilities, including agent-specific training. In the past 10 years, he has been responsible for the design, commissioning, and operations of 11 BSL-2 and BSL-3 facilities.

This report is based on a presentation given by John Lopez at Tradeline’s International Conference on Biocontainment Facilities in March 2006.

For more information, please contact: John Lopez, Major Projects Manager, Lovelace Respiratory Research Institute, 2425 Ridgecrest SE, Albuquerque, NM 87108, 505-348-9468, jlopez@lrri.org, www.lrri.org.

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Project Information

- Size: 9,500 sf
- Cost: $7.2 million
- Time: 17 months
- Building Function: aerobiology, virology, microbiology, necropsy, small animal and primate vivarium.
- Architect: Lord, Aeck & Sargent, Atlanta
- Autoclave: Getinge USA Inc., Rochester, New York
- Biosafety Cabinets: NuAire, Plymouth, Minnesota
- Fire Protection Engineer: CCRD, Houston, Texas
- General Contractor: Gerald Martin, Ltd., Albuquerque, New Mexico
- MEP Engineering: CCRD, Houston, Texas
- Structural Engineer: Waller-Davis Associates Inc., Norcross, Georgia
- Waste Reduction System: WR2 Waste Reduction Inc., Indianapolis, Indiana

Figure 1

The Lovelace Respiratory Research Institute’s new 9,500-sf aerobiology research laboratory was finished in 17 months at a cost of $7.2 million. The state-of-the-art laboratory will be used to study aerosolized pathogens potentially used in biological terrorism and for vaccine efficacy testing. The majority of the building is BSL-3 rated and features specialized research equipment designed to simulate varying levels of pathogenic exposure. Because the building handles multiple select agents and animal species and is used for vaccine research it is subject to intensive regulatory requirements and federal oversight. (Photo courtesy of Lovelace Respiratory Research Institute.)
Figure 2

The CVQx team used a pyramid-based model to identify the potential actions that might be executed for each component and system of the project. At the base are vendor qualifications, static component tests and factory inspections. Each progressive level of the pyramid depends on success of the prior levels. At the top of the pyramid are the dynamic, functional operational tests of active systems. Each step of the process is thoroughly documented at all levels. (Image courtesy of Lovelace Respiratory Research Institute.)

From EPA's Office of Pesticide Programs 12/19/06
www.epa.gov/pesticides

In This Update:
Web Page Available on the Prevention of Foot Spa-Associated Nail Infections

To help reduce the potential for skin infections associated with use of whirlpool foot baths in nail salons, EPA, jointly with the Centers for Disease Control and Prevention, now has information on its Web site about this issue. This information will inform the public about steps they can take to protect themselves from infections. It also provides guidance to nail salon owners and operators about proper disinfecting procedures. This fact sheet highlights clear instructions on proper use of disinfection products and a set of recommended cleaning and disinfecting procedures for salon owners and workers to follow. A model product label is included on the web site to help guide nail salon workers in the proper use of the disinfectant; it also highlights the importance of reading and following label directions.

The most important steps salon patrons can take to reduce the likelihood of an infection are:

1) Avoid shaving during the 24 hours before receiving a pedicure and do not have a pedicure if you have an open cut on your feet or lower leg area; this has been linked to skin infections.
2) Become familiar with a salon’s cleaning and disinfecting routine. The consumer should not be afraid to ask what steps are taken to make the foot bath safe. Specifically,
   a) An EPA-registered hospital disinfectant should be used after each customer.
   b) All parts of the foot bath should be disinfected, including the filter screen, piping and jets that cannot be seen, after each customer. The disinfectant should be run through the foot bath system according to product label directions (typically, for about 10 minutes).

The web page can be found at www.epa.gov/pesticides/factsheets/pedicure.htm
2006 ABSA Service Award Recipients

Award Presentations from the ABSA Conference, October 2006

Arnold G. Wedum
Distinguished Achievement Award
Maureen Ellis, MSc, RBP
Public Health Canada
Ottawa, Ontario, Canada

The Arnold G. Wedum Distinguished Achievement Award is given to a CURRENT ABSA member for outstanding contributions to biological safety accomplished through teaching, research, service, or leadership.

The award this year is presented to Maureen Ellis for numerous contributions to the field of biosafety through preparation and publication of official Canadian reports, guidance documents, and White Papers which have set the standards for documents created by other governments. In addition, she has participated in the work of the International Veterinary Association, the World Health Organization, and the International Biosafety Working Group. Maureen continues to contribute to the field as the Director of the Office of Biosafety at Health Canada.

Robert I. Gross
Memorial Award
Rebecca Colman, BS
Center for Microbial Genetics and Genomics
Northern Arizona University, Flagstaff, Arizona

Awarded to a student in recognition of academic achievement in biological safety.

Rebecca received her Bachelors of Science in chemistry and biology from Linfield College in McMinnville, Oregon in 2005. She is currently a PhD student under the co-advisement of Dr. Paul Keim and Dr. Dave Wagner at Northern Arizona University, where she has been studying the molecular diversity and epidemiology of Yersinia pestis for the past year.

Abstract: Yersinia pestis, the causative agent of plague, is a potential bioterrorism threat and a zoonotic disease that is endemic in the western United States; the most active focus is in the Four Corners region. Given these two different routes of infection (bioterrorism vs. natural source), it is crucial for public health investigators to be able to rapidly determine the source of an unknown plague infection. In 2002 a couple vacationing in New York came down with plague, which is not endemic to the east coast, raising the question: where did the couple contract plague? If the infection occurred in New York, one explanation would be that the couple contracted plague from a bioterrorism event. The couple made their home in Santa Fe County, New Mexico, where plague is endemic. Hence they may have contracted the infection at home. Therefore, with two possible sources, there was a need to use molecular epidemiology to match the infection to the source. We will review the initial public health investigation of this event, including the high-resolution DNA-fingerprinting system used to analyze the samples. Using this investigation as an example, we will describe new statistical and probabilistic methods that can be used to associate an unknown human isolate to a likely infection source. These methods represent important advancements for both forensic and public health investigators.

John H. Richardson
Special Recognition Award
Lynn Harding, MPH, CBSP
Biosafety Consultant
Chattanooga, Tennessee

The John H. Richardson Special Recognition Award was developed to recognize an individual for a specific contribution that has enhanced the American Biological Safety Association and/or the profession of biosafety.

This award is presented to Lynn Harding for her work in establishing the Certification Maintenance Program and, as head of the committee, developing and implementing the guidelines that bring professionalism to the area of biosafety. In addition, as the chair of the Awards Committee she oversaw fundamental changes in the procedures employed in selecting individuals whose service to the Association and the area of biosafety should be acknowledged by the Association.

Everett Hanel, Jr.
Presidential Award
Richard Rebar, MS, RBP, CBSP
GlaxoSmithKline
King of Prussia, Pennsylvania

The Everett Hanel, Jr. Presidential Award is given to a CURRENT ABSA member for outstanding contributions to ABSA by promoting the field of biological safety and fostering, by example, the high professional standards of the Association’s membership.

The award this year is presented to Richard Rebar for his outstanding service to ABSA. Richard has nearly 30 years of experience in basic and pharmaceutical research,
and has spent the last 15 years working in biosafety, radiation safety, and ergonomics. Richard has mentored numerous ABSA members and has served the Association as Councilor, Secretary, and as a Chairman and participant in many of the organization’s committees.

Richard C. Knudsen

Memorial Publication Award

Jay Krishnan, MSc
Public Health Agency of Canada
Winnipeg, Manitoba, Canada

The award shall be given, when merited, to the author(s) of an article that reports a significant contribution in scientific investigation and/or health and safety in areas of interest to Richard Knudsen during his career. The award recipient need not be a member of the American Biological Safety Association.


Mr. Krishnan, the first author of the article selected for the Richard Knudsen Memorial Publication Award, earned his MSc from the University of Manitoba. He is a Biologist doing applied biosafety research at the National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, where he has worked for the last 4 years.

This original research article was selected for its contributions to advances in biodecontamination. This research work evaluated vaporized hydrogen peroxide as an alternative to formaldehyde for large space decontamination.

Training Resource

The U.S. Department of Transportation provides a 36-slide Powerpoint training on “Transporting Infectious Substances Safely.” Changes in shipping regulations which took effect on October 1, 2006 are explained. The slides may be downloaded from http://hazmat.dot.gov/training/Transporting_Infectious_Substances_Safely.pdf

Packing and Marking of Category B

§173.199 Category B infectious substances.

Revised Required marking on outer package of Category B infectious substance adjacent to proper shipping name “Biological substances, Category B”

UN3373

Sample slide
New ABSA Members for 2007

Daniel Apple  
SAIC  
Alexandria, VA

Ghislain Beauregard  
Arcoplast, Inc.  
St. Peters, MO

Anthony Capps  
Sabre Technical Services  
Glen Ellyn, IL

Mireille Carter  
Boehringer Ingelheim (Canada) Ltd.  
Laval, Quebec, Canada

Patricia D. Cox  
Tetra Tech EMI  
Leeds, AL

Maria Douglass  
ImPart  
Falls Church, VA

David J. Driscoll  
Parkin Architects Limited  
Toronto, Ontario, Canada

Martha Gable  
Provincial Laboratory for Public Health  
Edmonton, Alberta, Canada

Claudia Gentry-Weeks  
Colorado State University  
Fort Collins, CO

Ayoob Ghalami  
University of Toronto  
Toronto, Ontario, Canada

R. Ross Graham  
DAI  
Woodbridge, VA

David Huizen  
Pfizer  
Kalamazoo, MI

Lewis Johnson  
Florida Gulf Coast University  
Ft. Myers, FL

Wai Kuen Kam  
Duke-NUS Graduate Medical School  
Singapore

Annette Ross Kemp  
Dugway Proving Ground  
Dugway, UT

Courtney Kerr  
Eastern Virginia Medical School  
Norfolk, VA

Steve Lesko  
Novartis Pharmaceuticals Corporation  
East Hanover, NJ

Sam Lipson  
Cambridge Public Health Department  
Cambridge, MA

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Boston, Massachusetts
Contact: www.animallab.com/turnkey

May 14-18, 2007
“Guidelines for Laboratory Design: Health and Safety Considerations” Conference
Harvard School of Public Health, Boston, Massachusetts
Contact: Phone: 617-384-8692, Fax: 617-384-8690, E-mail: contedu@hsph.harvard.edu,
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October 7-10, 2007
American Biological Safety Association (ABSA) 50th Annual Conference
Opryland Hotel, Nashville, Tennessee
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

October 19-22, 2008
American Biological Safety Association (ABSA) 51st Annual Conference
John Ascuaga’s Nugget, Reno, Nevada
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

October 18-21, 2009
American Biological Safety Association (ABSA) 52nd Annual Conference
Hyatt Regency Miami, Miami, Florida
Contact: Phone: 847-949-1517, Fax: 847-566-4580, E-mail: absa@absa.org, Web Site: www.absa.org

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