International
BIOLOGICAL THREAT REDUCTION

Principles of Laboratory Biosafety and Biosecurity

Laboratory Biosecurity and Biosafety
for BSL3 Laboratories
India
Jan 2007

www.biosecurity.sandia.gov

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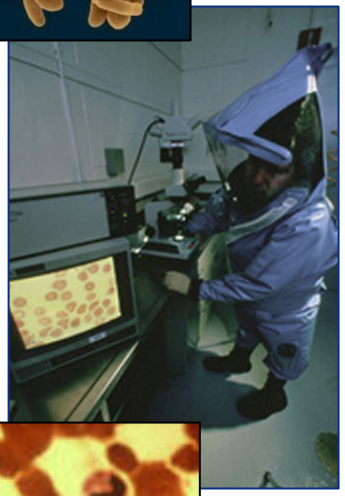
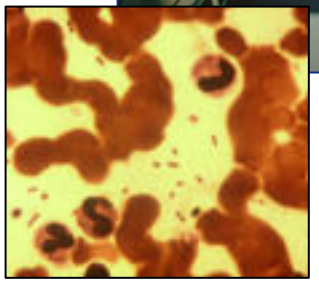
Sandia National Laboratories

Laboratory Biosafety and Biosecurity

- **Biosafety**
  - **Objective: reduce or eliminate accidental exposure to or release of potentially hazardous agents**
- **Biosecurity**
  - **Objective: protect biological agents against theft and sabotage by those who intend to pursue bioterrorism or biological weapons proliferation**
- **Common strategy**
  - **Implement graded levels of protection based on a risk management methodology**
- **Control of certain biological materials is necessary, but *how* that is achieved must be carefully considered**
  - **Biosecurity and biosafety should be integrated systems that avoid compromising necessary infectious disease research and diagnostics**

Francisella tularensis

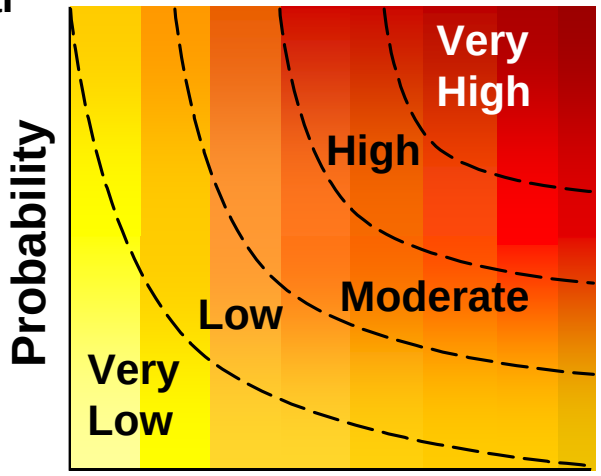




Yersinia pestis

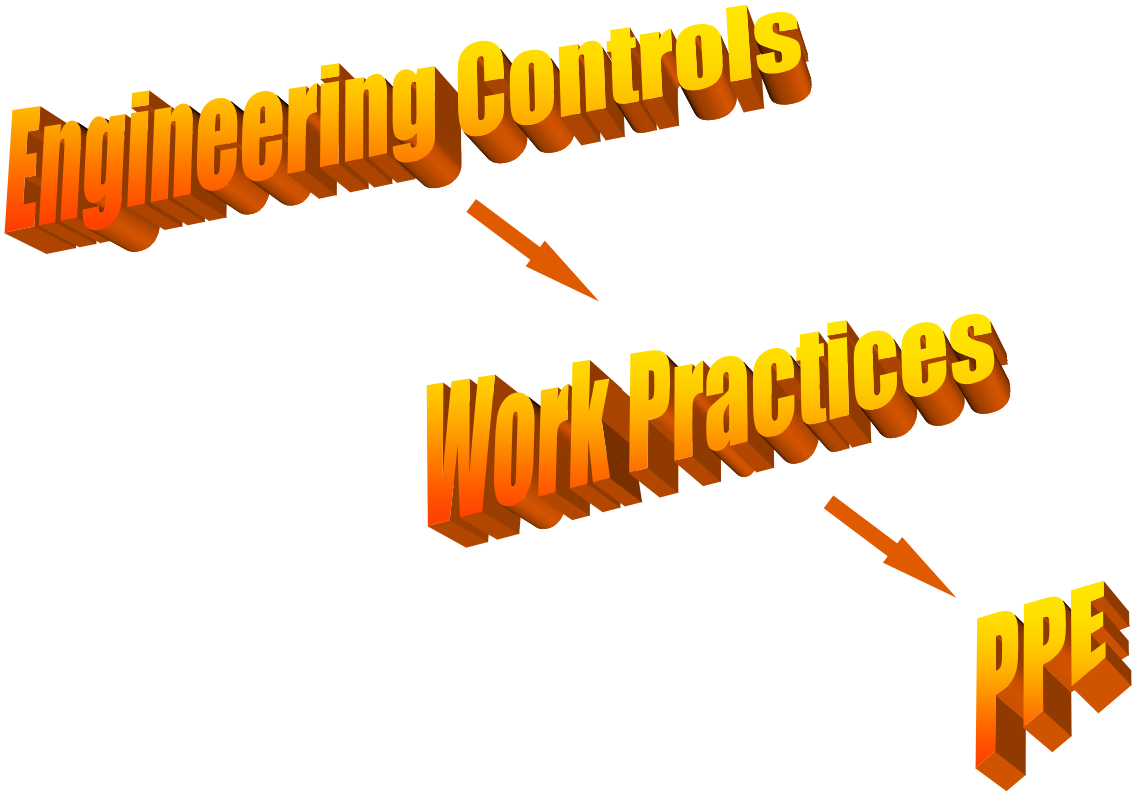
Laboratory Biosafety and Biosecurity Based on Risk Management

- Safety and security in a biological environment will never be perfect
- Most biological agents can be contracted or isolated from natural sources
- Critical not to compromise legitimate bioscience operations
- Management must distinguish between “acceptable” and “unacceptable” risks
  - Ensure that protection for an agent, and the cost, is proportional to the risk of accidental release/exposure or theft and misuse of that material



- Protect against unacceptable risk scenarios
- Develop incident response plans for acceptable risk scenarios

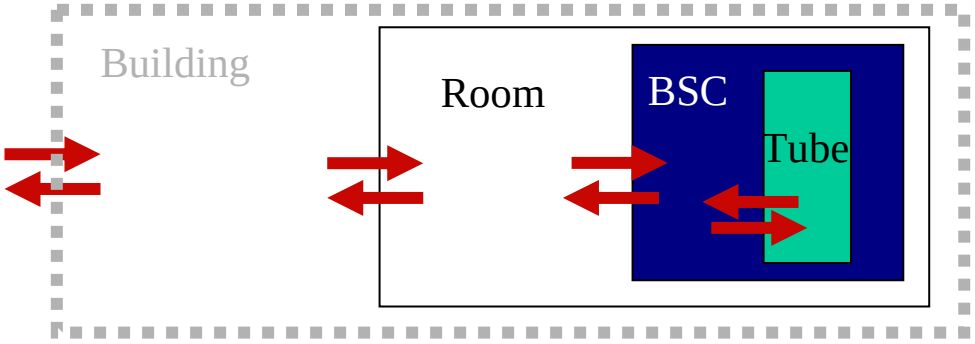
Components of Laboratory Biosafety



Biosafety: Engineering Controls

- **Primary barriers – contain the agent at the source**
  - Biological safety cabinet
  - Animal caging
  - Specialized lab equipment (centrifuges, fermenters, etc.)

- **Secondary barriers – contain the agent within the room or facility *in case an agent escapes from the primary barriers***
  - Building & Room Construction
  - HVAC Issues:
    - Directional airflow
    - Exhaust filtration
  - Other Engineering Controls:
    - Solid waste treatment
    - Wastewater treatment



Biosafety: Work Practices

- Good microbiological technique
- Wash hands often
- No mouth pipetting
- No eating or drinking in lab
- Minimize aerosol generation
- Careful pipetting technique
- Decontaminate work surfaces
- Safe sharps handling
- Training
- Written procedures

Biosafety: Personal Protective Equipment

- Types of PPE
  - Gloves
  - Appropriate footwear
  - Lab coats
  - Eye and face protection
  - Respirator
- Purpose
  - Provides a barrier against skin, mucous membrane or respiratory exposure to infectious agents
  - To prevent spread of contamination
- Limitations
  - Does not eliminate the hazard
  - Integrity wanes with use (change gloves frequently)
  - Not all gloves created equal – select best glove for the task

Biosafety Levels

- **Biosafety Level 1**
  - Suitable for work involving well-characterized agents not known to cause disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment.
  - Examples:
    - *Bacillus subtilis*
    - *Naegleria gruberi*
    - Infectious canine hepatitis virus
    - *E. coli*
- **Biosafety Level 2**
  - Suitable for work involving agents of moderate potential hazard to personnel and the environment
  - Examples:
    - Measles virus
    - Salmonellae
    - Toxoplasma species
    - Hepatitis B virus
- **Biosafety Level 3**
  - Suitable for work with infectious agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.
  - Examples:
    - *Mycobacterium tuberculosis*
    - St. Louis encephalitis virus
    - *Coxiella burnetii*
- **Biosafety Level 4**
  - Suitable for work with dangerous and exotic agents that pose a high individual risk of aerosol transmitted laboratory infections and life-threatening disease.
  - Examples:
    - Ebola Zaire virus
    - Rift Valley Fever virus

Biosafety Levels, Practices, and Equipment

Table 2. ***Relation of risk groups to biosafety levels, practices and equipment***

RISK GROUP	BIOSAFETY LEVEL	LABORATORY TYPE	LABORATORY PRACTICES	SAFETY EQUIPMENT
1	Basic – Biosafety Level 1	Basic teaching, research	GMT	None; open bench work
2	Basic – Biosafety Level 2	Primary health services; diagnostic services, research	GMT plus protective clothing, biohazard sign	Open bench plus BSC for potential aerosols
3	Containment – Biosafety Level 3	Special diagnostic services, research	As Level 2 plus special clothing, controlled access, directional airflow	BSC and/or other primary devices for all activities
4	Maximum containment – Biosafety Level 4	Dangerous pathogen units	As Level 3 plus airlock entry, shower exit, special waste disposal	Class III BSC, or positive pressure suits in conjunction with Class II BSCs, double-ended autoclave (through the wall), filtered air

BSC, biological safety cabinet; GMT, good microbiological techniques (see Part IV of this manual)

From: WHO LBM 3rd edition

Summary of Biosafety Level Requirements

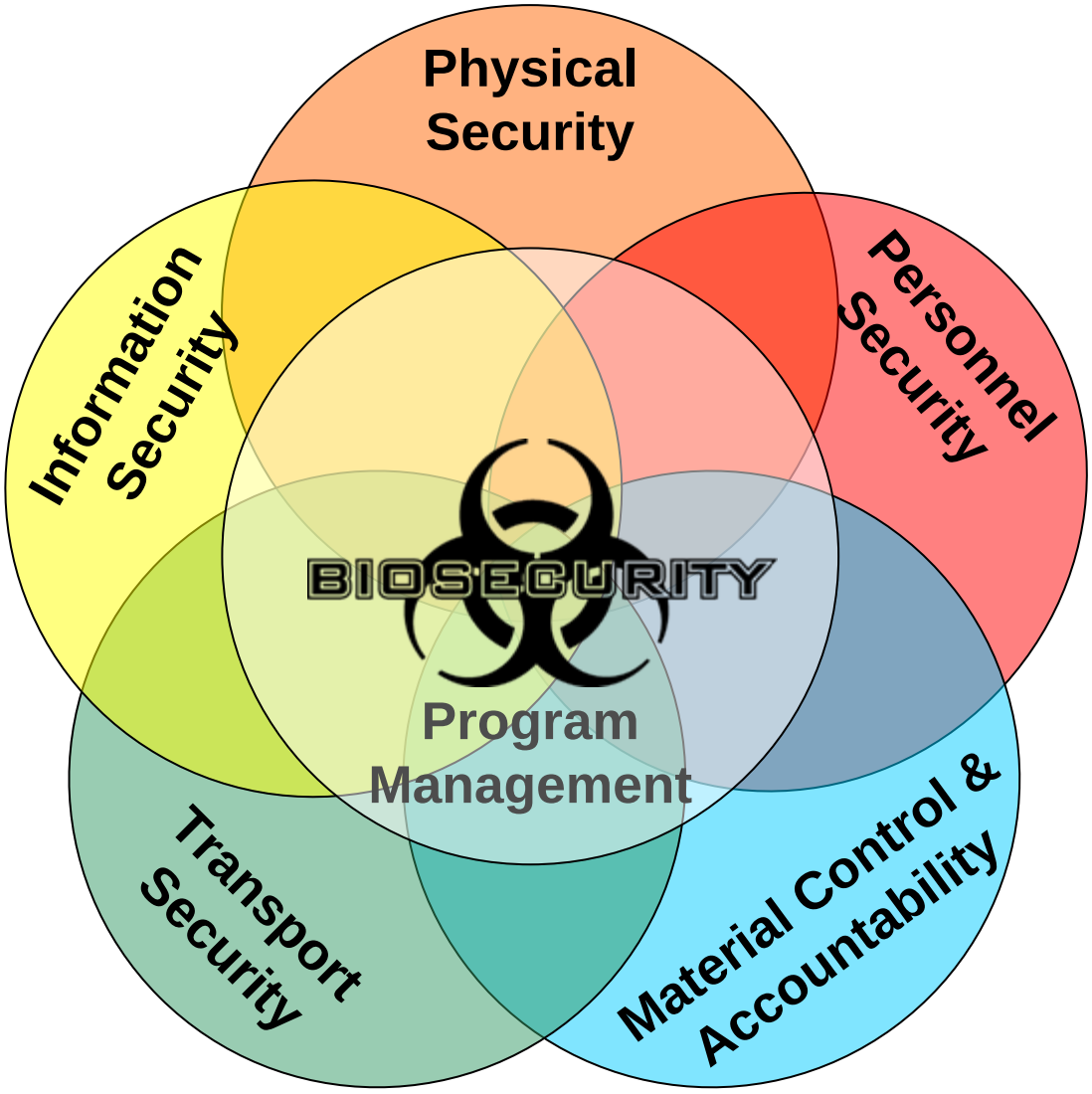
Table 3. ***Summary of biosafety level requirements***

	BIOSAFETY LEVEL			
	1	**2**	**3**	**4**
Isolation[a] of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation:				
— inward airflow	No	Desirable	Yes	Yes
— controlled ventilating system	No	Desirable	Yes	Yes
— HEPA-filtered air exhaust	No	No	Yes/No[b]	Yes
Double-door entry	No	No	Yes	Yes
Airlock	No	No	No	Yes
Airlock with shower	No	No	No	Yes
Anteroom	No	No	Yes	—
Anteroom with shower	No	No	Yes/No[c]	No
Effluent treatment	No	No	Yes/No[c]	Yes
Autoclave:				
— on site	No	Desirable	Yes	Yes
— in laboratory room	No	No	Desirable	Yes
— double-ended	No	No	Desirable	Yes
Biological safety cabinets	No	Desirable	Yes	Yes
Personnel safety monitoring capability[d]	No	No	Desirable	Yes

[a] Environmental and functional isolation from general traffic.
[b] Dependent on location of exhaust (see Chapter 4).
[c] Dependent on agent(s) used in the laboratory.
[d] For example, window, closed-circuit television, two-way communication.

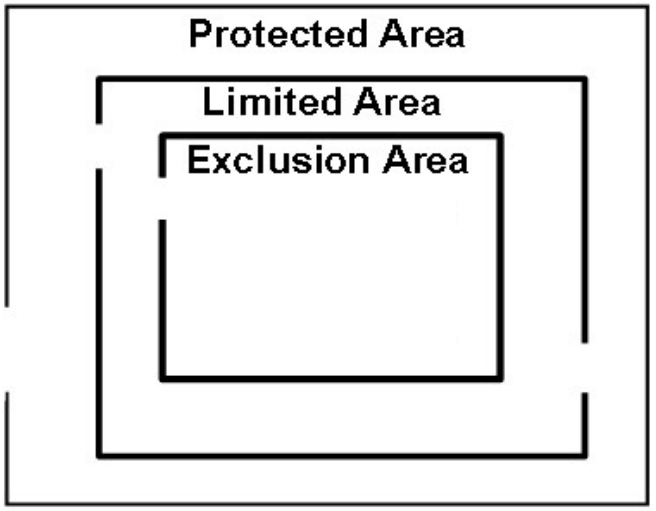
From: WHO LBM 3rd edition

Components of Laboratory Biosecurity



Biosecurity:
Physical Security and Personnel Security

- **Physical security – restricts access to authorized individuals**
  - **Defined perimeter**
  - **Access controls**
  - **Intrusion detection**
  - **Response**
- **Personnel security – determines who is authorized**
  - **Personnel screening (background checks)**
  - **Badges**
  - **Visitor control**

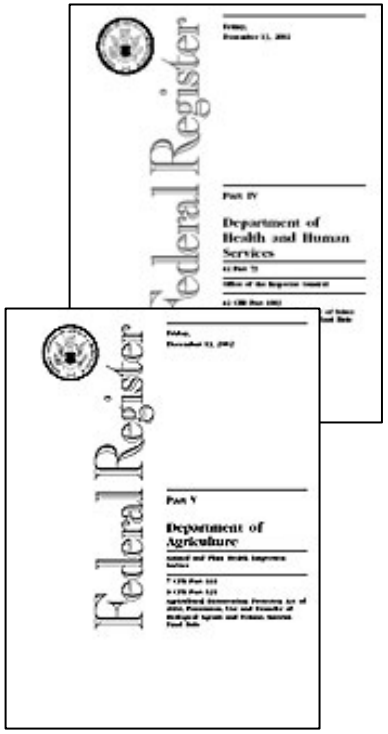


Biosecurity: MC&A, Transport Security, Information Security

- **Material Control & Accountability (MC&A) – provides awareness of what materials exist where and who is responsible**
  - **Physical and procedural controls**
  - **Inventories**
  - **Accountable individuals**
- **Transport security – MC&A for materials being transferred between laboratories**
  - **Knowledge of recipient**
  - **Physical security of packages**
  - **Personnel screening for individuals who handle packages**
  - **Chain of custody**
  - **Use of reliable carriers**
- **Information security – protecting sensitive information from public release**
  - **Identification, marking, and control**
  - **Network and communication security**

US Select Agent Rule (2005)

- Facility registration if it possesses one of 80 Select Agents
- Facility must designate a Responsible Official
- Background checks for individuals with access to Select Agents
- Access controls for areas and containers that contain Select Agents
- Detailed inventory requirements for Select Agents
- Security, safety, and emergency response plans
- Safety and security training
- Regulation of transfers of Select Agents
- Extensive documentation and recordkeeping
- Safety and security inspections



Heightened Security or Neocolonial Science?

New restrictions on federally funded research involving the world's most dangerous pathogens are hampering foreign collaborations

ALMATY, KAZAKHSTAN—Scott Weaver thought he had a green light for a great research partnership. After an expensive security upgrade of his labs and hours of paperwork, the director for tropical and emerging infectious disease research at the University of Texas Medical Branch (UTMB) in Galveston was ready to resume research on the Venezuelan equine encephalitis (VEE) virus in Colombia, Peru, and Venezuela. The mosquito-borne disease, endemic in all three countries, is not the worst of its kind: The alphavirus kills less than 1% of its human victims. But VEE's potential to incapacitate has landed it on a list of "select agents": several dozen of the nastiest sorts of pathogens that the U.S. government fears could be turned into biological weapons. That designation has thrown up new hurdles for Weaver and his collaborators in South America—and for many other U.S. scientists working overseas.

In August, the U.S. National Institute of Allergy and Infectious Diseases (NIAID) informed Weaver that under the terms of his two VEE grants, the laboratories of his foreign colleagues must have procedures in place for handling select agents that are equivalent to tough U.S. regulations* imposed last year. "I seriously doubt whether my collaborators in Caracas or Bogotá could ever meet U.S. standards for select-agent security," says Weaver. "These developing countries cannot afford the kinds of elaborate systems that labs in the U.S. have been required to install," such as sophisticated security and inventory systems and background checks on employees. He's since had to alter his projects to avoid isolating the VEE virus in the labs south of the border. Because the new policy may force some foreign partners to serve as mere sample exporters, it resurrects "the stereotype of the ugly American: arrogant, demanding, and insensitive," Weaver charges: "American collaborations will be unwelcome in many developing countries of the world."

Although his case may be one of the first, Weaver is not the only researcher feeling the chill. According to a prominent U.S. specialist on select agents, researchers with the U.S. Centers for Disease Control and Prevention (CDC) have seen a curtailment of foreign collaborations on avian flu and viral hemorrhagic fevers. (CDC officials declined to comment.) Scientists at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Frederick, Maryland, are experiencing similar constraints on projects involving Congo-Crimean hemorrhagic fever and related diseases. "The important work we need to do will get done," says USAMRIID public affairs officer Caree Vander Linden, although the details have not been worked out.

No picnic. Venezuelan scientists draw blood from rodents to isolate VEE virus. New NIH rules have crimped projects on this and other select agents.

U.S. inspectors will soon be heading out to assess lab standards overseas, scientists learned at a closed-door meeting last month. Paula Stricklland, acting director of NIAID's Office of International Extramural Activities, told a group at the annual meeting of the American Society of Tropical Medicine and Hygiene (ASTMH) in Miami, Florida, that security teams will include senior microbiologists from CDC's select-agents program. An interagency committee chaired by Stricklland with representatives from the U.S. State and Justice departments will determine whether foreign labs "meet minimum biosafety and biosecurity requirements."

The stepped-up regulations are the latest example of the clash between scientists' cherished ways of doing business and the urgent need to reduce the potential for bioterrorism, and some researchers say the rules make sense. "It would be very embarrassing for a U.S. collaborator and a U.S. agency to be funding a facility that had a major accident, or one that was involved in a bioterrorism event," says Paul Keim, an anthrax specialist at Northern Arizona University in Flagstaff.

But others fear that the tightened security could stifle cooperation. "One doesn't develop productive collaborative relationships with foreign counterparts by announcing upon arrival that 'from now on we must do things the American way,'" says UTMB arbovirus specialist Robert Tesh. "Each country has its security priorities. The U.S. cannot demand that they conform to ours."

Adds Weaver: "By inhibiting research on the ecology and epidemiology of potential biological weapons in their natural settings overseas, we will be less prepared to respond optimally to the introduction of these agents by a terrorist."

Clampdown

After letters containing powdered anthrax were mailed to members of Congress and others in the fall of 2001, the U.S. government crafted tough requirements for scientists it funds to study dangerous pathogens. In addition to tightening security at facilities in which the microbes are kept and studied, U.S. regulations now demand rigorous protocols covering security assessments, emergency response plans, training, transfers of materials, and inspections.

Under the new NIAID rules, which the institute began developing in 2003, U.S. grantees must submit a dossier on a foreign collaborating institution detailing its "policies and procedures for the possession, use, and transport of select agents." For what NIAID calls "security risk assessments," grantees "must be willing to provide the names of all individuals who will have access to the select agents."

Weaver says the new rules prompted him to drop his original plan to process field samples potentially infected with VEE virus in South America. Now, he says, he will have all the samples shipped to Galveston. "This seems to have gotten me off the hook for the time being," he says, in that his colleagues at the National Institute of Health in Bogotá and the Central University of Venezuela and the National Institute of Hygiene in Caracas now won't have to adhere to the select-agent terms. But the change will reduce efficiency and timeliness, he says.

"Basically, the NIH [U.S. National Institutes of Health] left me with little choice," because it would have taken "months or years" to bring overseas labs into compliance, Weaver says. Already, the labs in Colombia and Venezuela store many VEE virus isolates in their freezers: Preventing the isolation of a few more strains, he says, will not deny the virus to a potential terrorist.

Although security at foreign facilities working with select agents generally has been strengthened since the 9-11 attacks, most labs would still run afoul of the new U.S. rules. Many outside the United States appear to be unaware of the regulations. "I haven't heard much," says Lev Sandakhchiev, director general of the State Research Center of Virology and Biotechnology, a former biowespons lab near Novosibirsk, Russia, that collaborates with the United States on smallpox research.

Foreign researchers say they hope to find a way to continue working with U.S. counterparts because it would bolster security in their home countries. "If collaborations will continue, that will inevitably bring the standards up," says Bakyt Atshabar, director of the Kazakh Science Center for Quarantine and Zoonotic Diseases in Almaty, Kazakhstan, which specializes in studying endemic plague with Pentagon funding (*Science*, 17 December, p. 2021).

ASTMH and other societies intend to lobby for a relaxation of the rules. "The approach to this will not be easy," says Peter Weller, an immunologist at Harvard Medical School in Boston and ASTMH's most recent past president. For one, many agencies will want to weigh in on any change of policy. Second, Weller says, "the facile reply is that you scientists gave the Pakistanis nuclear secrets; how do we trust you on these issues?" In an e-mail response to questions from *Science*, NIAID officials say they expect no change to the select-agent terms "in the immediate future."

But some experts such as Keim say raising global security levels to U.S. standards makes sense. "We should not allow U.S. researchers to avoid regulatory oversight by going abroad. This would certainly apply to human subjects in clinical trials and animal-care standards in animal protocols. Why not security of dangerous pathogens?"

Critics of the policy say they are not opposed to strengthened security overseas. Rather, they decry how the U.S. government is going about it. NIH "seems to be hell-bent on enforcing the regulations," says Thomas Monath, chief scientific officer at Acambis in Cambridge, Massachusetts, and president of ASTMH. He wonders whether his company's research on Japanese encephalitis, a select agent, with colleagues in Thailand and Australia will be subject to such oversight. Monath fears that U.S. researchers might be held criminally responsible for violations by collaborators. When he raised this issue with Stricklland at the ASTMH meeting, he says, it was apparent that "NIH had neither thought about this nor had any clear response."

NIAID officials say they are simply in step with the times; later they plan to adopt standards being developed by the World Health Organization. "We will do what we can to ensure that every possible avenue has been pursued that will allow our NIH-funded researchers to be able to conduct their research safely and securely," the officials say. Much of that work, it appears, may well have to be done inside U.S. borders.

–RICHARD STONE

* www.cdc.gov/od/sap/docs/42cfr73.pdf

A Selection of Select Agents

- Smallpox virus
- Crimean-Congo hemorrhagic fever virus
- Lassa fever viruses
- Central European tick-borne encephalitis
- *Yersinia pestis* (plague)
- Foot-and-mouth disease virus
- Ebola viruses
- Ricin
- Tetrocotoxin
- *Bacillus anthracis* (anthrax)
- Venezuelan equine encephalitis virus
- Botulinum neurotoxin

Some Countries Are Betting That A Few Seconds Can Save Lives

Japan, Mexico, and Taiwan are investing in early warning systems that can offer precious seconds of warning before a major tremor

TOKYO—What would you do with 5 to 50 seconds' warning of a major earthquake?

It's not an academic question. Systems that can detect earthquakes near their source and issue warnings before the shaking starts are in place or being deployed in Mexico, Taiwan, and Japan and are being studied for locales from southern California to Istanbul. Enthusiasts are convinced that short-term warnings can save lives by stopping trains before they pass over damaged track, emptying out elevators, and alerting rescue units. "It is an epochmaking" advance in earthquake safety, says Masato Motosaka, a Japanese earthquake engineer at Tohoku University in Sendai.

On alert. Nowcast stations are being installed across Japan.

Not everyone agrees, however. Skeptics note that warning systems don't provide enough time to reduce casualties close to the epicenter of an earthquake. They also worry that such systems could divert spending from earthquake preparedness, which they say has the potential to do much greater good. "Warnings only help in some cases," says Robert Olshansky, an urban planner at the University of Illinois, Urbana-Champaign. "Investing too much of one's money and hopes in a short-term warning system is a distraction from the hard and less sexy work, such as upgrading older structures, that is really needed to improve seismic safety."

Faster than a speeding S wave

Early warning systems are not forecasts. Instead, they detect actual quakes near their source and issue warnings to automated systems and humans up to several hundred kilometers away. They work because electronic signals transmitted through wires or air travel faster than seismic waves moving through the earth. Warning schemes also take advantage of the two types of seismic waves that are generated when a fault ruptures. The first—and faster moving—primary (P) waves radiate directly outward from the epicenter. The secondary (S) waves, which cause the oscillating motions responsible for the most damage, lag by tens of seconds over a distance of a few hundred kilometers. "The P waves carry information; the S waves carry energy," explains Hiroo Kanamori, a seismologist at the California Institute of Technology (Caltech) in Pasadena. Unfortunately,

Biosecurity Goes Global

The 2001 anthrax letters triggered a strong U.S. response. Now the rest of the world is starting to take biosecurity more seriously—but not necessarily by adopting the U.S. approach

Three years ago, the small number of life scientists using the term "biosecurity" were talking about ways to keep diseased crops and livestock from crossing national borders. Then came the fatal October 2001 anthrax letter attacks against several U.S. targets. In short order, thousands of U.S. scientists were confronted with an avalanche of new and often unpopular rules designed to keep potentially dangerous pathogens and toxins away from bioterrorists. Researchers who break those rules could face significant criminal penalties.

Despite these aggressive steps on the home front, U.S. officials readily acknowledged that unilateral action was insufficient and that the world needed to form a united front against increasingly sophisticated biotechnologies. But many nations were skeptical of the threat. They also doubted the value of what critics call "the guns, guards, and gates" approach to biosecurity. The result, says Reynolds Salerno, a biosecurity expert at Sandia National Laboratories in Albuquerque, New Mexico, has been "tremendous confusion and concern in the international life sciences community about biosecurity."

That confusion may be giving way to cooperation, however, as an increasingly global effort to define and implement biosecurity is gaining speed. Nations are moving to pass new biosecurity laws, while public health and security experts are hammering out voluntary biosecurity guidelines and debating "codes of conduct" for life scientists. Many countries are thinking about looser rules for less risky agents than in the United States, which critics say has imposed a one-size-fits-all approach, and few are likely to require the extensive criminal background checks carried out by U.S. agencies.

The new world order may not resemble the U.S. model. But like it or not, life scientists worldwide are about to become much more familiar with the term biosecurity.

–David Malakoff

Science, Vol. 305, 17 September 2004

The BWC and Biosecurity

- Bacteriological (Biological) and Toxin Weapons Convention (BWC) addresses three relevant issues
  - National Implementing Legislation
  - National Pathogen Security (biosecurity)
  - International Cooperation

- Recent technical experts meetings to strengthen the BWC.
  - States Parties agree to pursue national implementation of laboratory and transportation biosecurity (2003)

UNSCR 1540 and Biosecurity

- Urges States to take preventative measures to mitigate the threat of WMD proliferation by non-state actors
- UNSCR 1540 requires States to
  - Establish and enforce legal barriers to acquisition of WMD by terrorists and states
  - Submit reports to the 1540 Committee on efforts to comply
- Paragraph 3 is the key provision that supports biosecurity
  - "Take and enforce effective measures to establish domestic controls to prevent the proliferation of . . . biological weapons . . .; including by establishing appropriate controls over related materials"
    - Develop and maintain appropriate effective measures to account for and secure such items in production, use, storage or transport
    - Develop and maintain appropriate effective physical protection measures

Laboratory Biosecurity Supports Laboratory Biosafety

- **Laboratory biosecurity supports the laboratory biosafety agenda of preventing disease in people, animals, and plants and minimizing the risk of worker injury**

- **Safe and secure laboratories help**
  - **Ensure the containment of hazardous infectious substances in laboratories**
  - **Maintain citizens' confidence in the activities of the bioscience research community**
  - **Increase transparency to investors in the biomedical and biotechnology industries**
  - **Protect valuable research and commercial assets**
  - **Reduce the risks of crime and bioterrorism**

Conclusions

- Biosafety has historically been based on guidance and best practices
- Biosecurity is much newer and regulations, guidelines, and implementation methodologies are evolving
- The “internationalization” of laboratory biosecurity practices is an important development
  - Securing dangerous pathogens in one or a few countries is insufficient to mitigate the threat of bioterrorism or biological weapons proliferation
- However, the US Select Agent Rule is not universally applicable
  - Laboratory biosecurity guidelines and requirements need to reflect local and national concerns and priorities

“Infectious diseases make no distinctions among people and recognize no borders”
President George Bush, November 2001

Resources

- **Laboratory Biosafety and Biosecurity Guidance – provided on CD**
  - **WHO Laboratory Biosafety Manual, 3rd edition (Ch 9 is Laboratory Biosecurity)**
    - **WHO/FAO/OIE developing joint guidance, working title – Biorisk management: Laboratory Biosecurity Principles**
  - **CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories***
    - **4th edition is the current edition (on CD), 5th edition forthcoming in 2006 which will include extensive recommendations on biosecurity**
  - **Canada's Laboratory Biosafety Guidelines, 3rd edition**

- **Transport of Infectious Substances**
  - **IATA guidance**
  - **WHO guidance – provided on CD**

- **On the Web**
  - **Asia-Pacific Biosafety Association: www.a-pba.org**
  - **American Biological Safety Association: www.absa.org**
  - **Sandia National Laboratories: www.biosecurity.sandia.gov**