BSL-3 LABORATORY PRACTICES IN THE UNITED STATES: COMPARISON OF SELECT AGENT AND NON-SELECT AGENT FACILITIES

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New construction of biosafety level 3 (BSL-3) laboratories in the United States has increased in the past decade to facilitate research on potential bioterrorism agents. The Centers for Disease Control and Prevention inspect BSL-3 facilities and review commissioning documentation, but no single agency has oversight over all BSL-3 facilities. This article explores the extent to which standard operating procedures in US BSL-3 facilities vary between laboratories with select agent or non-select agent status. Comparisons are made for the following variables: personnel training, decontamination, personal protective equipment (PPE), medical surveillance, security access, laboratory structure and maintenance, funding, and pest management. Facilities working with select agents had more complex training programs and decontamination procedures than non-select agent facilities. Personnel working in select agent laboratories were likely to use powered air purifying respirators, while non-select agent laboratories primarily used N95 respirators. More rigorous medical surveillance was carried out in select agent workers (although not required by the select agent program) and a higher level of restrictive access to laboratories was found. Most select agent and non-select agent laboratories reported adequate structural integrity in facilities; however, differences were observed in personnel perception of funding for repairs. Pest management was carried out by select agent personnel more frequently than non-select agent personnel. Our findings support the need to promote high quality biosafety training and standard operating procedures in both select agent and non-select agent laboratories to improve occupational health and safety.

IN RECENT YEARS, the number of biosafety level 3 (BSL-3) facilities has increased to facilitate research on potential bioterrorism agents.^{1,2} Increased budgets (approximately \$50 billion) have been reported (2001-2008) for agencies involved in bioterrorism-related research, including the Department of Defense (DOD), the Department of Health and Human Services (HHS), and the Department of Homeland Security (DHS).³ The US Government Accountability Office (GAO) conducted a study in 2009 to assess risks associated with

containment laboratories with regard to prevalence, tracking, and regulation.⁴ The study showed that oversight improves biosafety, and it highlighted a need for individual laboratories to implement and evaluate personnel monitoring systems. Homeland Security Presidential Directives 10 and 18 (HSPD-10 and -18) earmarked federal funding to construct new containment laboratories—for example, the National Institute of Allergy and Infectious Diseases (NIAID) started 13 BSL-3 facilities.^{1,5-8} This spurred NIAID to fund a 2-year fellowship

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training for biosafety professionals in 2004 that included training for maintenance workers. Currently, there is a National Biosafety and Biocontainment Safety Program fellowship funded by NIAID and the Division of Occupational Health and Safety that provides opportunities for post-baccalaureates to receive 2 years of professional biosafety training.⁹

In 2005, 2 separate surveys were carried out by the National Institutes of Health (NIH) and HHS to determine the operational status of BSL-3 laboratories in the United States. Respective surveys showed 292¹⁰ and >600¹¹ operational BSL-3 laboratories. In 2007, 1,356 BSL-3 laboratories were registered with the Centers for Disease Control and Prevention (CDC) or Animal and Plant Health Inspection Service of the US Department of Agriculture (APHIS-USDA) for select agent research.¹ In 2007, the GAO noted that no single government agency was responsible for risk assessment and oversight of all BSL-3 laboratories, although the report did not specify if clinical BSL-3 laboratories were included.¹ The number of BSL-3 facilities in the US is currently unknown because federal registration is required only if select agent (National SA Registry) or NIH-funded recombinant DNA (rDNA) (Institutional Biosafety Committee [IBC]) work is conducted.^{1,12,13} Laboratories conducting NIH-funded research are required to have an NIH-registered IBC. BSL-3 work that does not involve rDNA is not required to be registered with the IBC. rDNA work that is not funded by NIH is required to have IBC oversight, but not federal registration.

Laboratory-Acquired Infections

The risk of a laboratory-acquired infection at any biosafety level is difficult to measure for several reasons, including but not limited to the lack of a national systematic reporting system¹⁴ and unwillingness to report laboratory-acquired infections. Select agent regulations (2 CFR §73.19)¹⁵ state that laboratories and/or individuals are required to report theft, loss, or release of select agents. A comprehensive survey of published laboratory-acquired infections reported that investigators were likely unwilling to report laboratory-acquired infections because of embarrassment or fear of dismissal.^{16,17} The first published report regarding incidence of laboratory-acquired infections was based on mail surveys and published literature.¹⁸ A later study examined laboratory-acquired infections using mail surveys, published literature, and personal communication and noted that 64% of laboratory-acquired infections had been officially reported.¹⁹ There is currently no centralized national database for laboratory-acquired infections;²⁰ hence, there is no system to evaluate safety measures.

Biosafety Level 3

According to the Biosafety in Microbiological and Biomedical Laboratories (BMBL) guidelines, workers in BSL-3 laboratories should be under medical surveillance and

provided relevant vaccines (if available).²¹ BSL-3 laboratories performing NIH-funded research must adhere to established requirements, including administrative and engineering controls, personal protective equipment, decontamination procedures, lab safety, and security.^{21,22}

Select Agents

US facilities working with, possessing, or transferring biological agents or toxins that pose a “severe threat to public, animal or plant health, or to animal or plant products” are required to register with the National SA Registry.¹⁵ Select agent facilities carry out risk assessments, provide safety and training records, create emergency response plans, and document pathogen transfers and usage. Select agent facilities are subject to unannounced inspections. A temporary shutdown at Texas A&M University in 2006 resulted from failure to disclose a laboratory-acquired infection when a non-select agent worker was exposed to a select agent (*Brucella*) and experienced clinical symptoms of illness. In 2007, other potential exposures to *Coxiella burnetii* (based on serum titers) contributed to the shutdown because of the federal requirement to report select agent incidents.⁴

Laboratory Structure and Maintenance

The maintenance of BSL-3 laboratories is especially important, as structural cracks can compromise decontamination procedures and/or allow infiltration by pests such as insects that may be mechanical pathogen vectors. In 2007, an outbreak of foot-and-mouth disease occurred at the Institute for Animal Health in England, leading to the slaughter of more than 2,100 animals at neighboring farms.²³ This case showed that pathogens likely leaked through wastewater treatment pipes attached to the facility.²⁴

Personnel Training

Biosafety training is needed to ensure the safety of all workers.^{1,25} General laboratory training instructs workers on biosafety principles, but specialized training is required for personnel working at higher BSLs. A “mentor-apprentice” relationship is used, with the trainee moving up in BSL work as he or she acquires experience.⁶ However, the extent of this practice may vary among laboratories. In 2008, an American Association for the Advancement of Science workshop determined that additional funding for training and maintenance would reduce risk at biocontainment facilities.²⁶

Recombinant DNA

In the United States, institutions with any rDNA research must establish an Institutional Biosafety Committee (IBC) to determine the BSL, facilities, procedures, and expertise/

training required for each experiment.¹³ The nature of rDNA work poses unknown risks and involves the insertion of a DNA sequence of one organism into the DNA of another organism.

The number of BSL-3 laboratories has increased since 2001. Consequently, the objective of this study was to determine the extent to which variation exists in standard operating procedures between select agent and non-select agent facilities in the United States.

METHODS

Through a Freedom of Information Act request, the list of NIH-registered IBCs was obtained. Non-US IBCs were removed, leaving 754 IBCs. We removed 61 IBCs due to lack of response and 264 IBCs due to lack of BSL-3 laboratories. Six IBCs without operational BSL-3 facilities and 2 new facilities that were not yet operational were also excluded. At 36 facilities, multiple IBCs were affiliated with the same facility. Three IBCs with BSL-3 and 1 with BSL-3 and BSL-4 facilities elected not to participate. The remaining 381 contacts were sent a reminder e-mail 2 weeks before the close of the survey. The final pool was 359 potential BSL-3 respondents.

Both the initial contact and the follow-up reminder instructed participants to complete the survey administered through the web-based survey tool SurveyMonkey (East Carolina University Institutional Review Board Approval #11-0549). This allowed for increased anonymity in survey responses, and a password system prevented anyone other than the IBC contact from adding false or duplicate data. A SurveyMonkey filtering tool blocked multiple responses from the same IP address. The survey remained open from September 24 through November 8, 2011.

Statistical Analyses

Five facility types were surveyed: academic, nonprofit, state, federal, and private. Respondents were permitted to choose only 1 type when classifying their institution. Funding status was self-reported as “adequate” or “inadequate,” and select agent status was also self-reported. Most facilities started operation in the 1990s and 2000s, so only data from these decades were compared. All statistical comparisons were made between select agent and non-select agent facilities.

Fisher exact tests ($\alpha=0.05$) (SPSS 2010, Somers, NY) were used to evaluate the extent to which select agent and non-select agent facilities differed in the following areas: personnel training, decontamination, PPE, medical surveillance, security access, laboratory structure and maintenance, funding, and pest management. Although data are presented about collection of baseline serum samples, caution is advised when interpreting comparisons since baseline serum collection is optional and based on individual risk assessment.

RESULTS

Characteristics of Responding Institutions

There were 93 of 359 (26%) responding institutions encompassing all biosafety levels except for BSL-4. There were 60 of 359 responses (16% response rate) from BSL-3 facilities. Types of respondents were: 72% (43/60) academic, 17% (10/60) nonprofit, 5% (3/60) state, 3% (2/60) federal, and 3% (2/60) private. Twenty facilities (48%) self-identified as select agent, and 22 (52%) facilities were non-select agent; hence, these 42 facilities were used for all analyses.

Personnel Training

Significant differences were observed between select agent and non-select agent facilities in 4 surveyed training components: hands-on, written, lecture, and competency assessment. Facilities working with select agents were more likely to use all 4 components, while non-select agent facilities used only 3 training components (hands-on, lecture, and competency assessment). In select agent (13/20, 65%) but not non-select agent (10/22, 45%) facilities, most nonscientific staff working in areas near research laboratories were trained to respond to an accidental pathogen release. The level of personnel training is expected to vary between facilities, depending on the type of pathogen research.

Decontamination and Waste Transport

Significant differences were observed between select agent and non-select agent facilities ($P=0.008$) in decontamination techniques: autoclave; autoclave + chemical; and autoclave + chemical + incineration. We observed that select agent facilities were more likely to use a combination of autoclave and chemical decontamination, while non-select agent facilities primarily used autoclaving. Neither select agent (15/20, 75%) nor non-select agent (20/22, 91%) facilities commonly transport materials outside of the laboratory for decontamination. Select agent facilities decontaminated (ie, wiped down) the entire lab either less than biennially or only after an accident, while non-select agent facilities were more likely to be decontaminated every 6 months or annually ($P=0.043$). As expected, both select agent and non-select agent facilities primarily use hands-free sinks (15/20 select agent, 75%; 21/22 non-select agent 95%).

Nineteen select agent and all 22 non-select agent respondents answered the questions about high-efficiency particulate air (HEPA) filters for the exhaust of facilities and frequency of complete facility decontamination. These HEPA filters were replaced every 6 months to 2 years at 6 of 19 (32%) select agent and 9 of 22 (41%) non-select agent facilities. The HEPA filters were replaced less frequently (at intervals more than every 2 years) at 13 of 19 (68%) select

agent and 13 of 22 (59%) non-select agent facilities. It should be noted that HEPA filters are not required to be changed regularly under all BSL-3 circumstances.

Personal Protective Equipment

Select agent facilities relied more on disposable PPE compared to non-select agent facilities ($P=0.003$). Eight select agent (40%) and 19 (86%) non-select agent respondents used both disposable and reusable PPE, while 12 (60%, select agent) and 3 (14%, non-select agent) used disposable PPE only. All institutions used disposable gloves, but both select agent (16/20, 80%) and non-select agent (16/22, 73%) facilities used nitrile more often than latex or vinyl gloves. Nineteen select agent and 18 non-select agent respondents answered the survey question about respiratory PPE. In select agent laboratories, workers use N95 respirators (8/19, 42%), N100 respirators (1/19, 5%), or powered air purifying respirators (PAPRs) (10/19, 53%) with either a full hood or a face shield. In contrast, non-select agent workers used only N95 respirators (14/18, 78%) and PAPRs (4/18, 22%).

Medical Surveillance

Medical surveillance was carried out primarily for scientific and support staff (not nonscientific staff) in both select agent and non-select agent facilities. Baseline and annual follow-up serum samples were collected from scientific staff only at 12 of 20 (60%) select agent and 8 of 22 (36%) non-select agent facilities.

Security Access

Forty respondents answered the survey question about security access. Entry into 85% (17/20) of select agent and 35% (7/20) of non-select agent facilities was by personal access code, while access to others was general (eg, key).

Laboratory Structure and Maintenance

There were no significant differences in reported structural integrity (eg, breaks, holes, open seams in walls, floors, or ceiling) between select agent and non-select agent laboratories. Two non-select agent facilities (2/22; 9%) and no select agent reported breaks, holes, or open seams in laboratory walls, flooring, or ceiling. One of the aforementioned non-select agent facilities self-identified as being not currently operational.

Funding

Significant differences ($P=0.013$) were observed between personnel at select agent and non-select agent facilities in

their perception of laboratory repairs. Most (15/20, 75%) select agent respondents reported that finding funding for these repairs was more difficult than arranging the repair. The opposite was true for non-select agent laboratories, where most (14/22, 64%) respondents believed that arranging the repair was more difficult than funding.

Pest Management

Pest management was carried out by select agent staff significantly more frequently ($P=0.00002$) than non-select agent staff, which contracted commercial pesticide applicators for this service.

DISCUSSION

Although the increased number of BSL-3 laboratories in the past decade has advanced infectious disease research, there are also potential public health risks.² A study recommended that policymakers consider the following options with regard to oversight of high-containment facilities:

- no change to existing policies;
- delay policy decisions pending investigation by groups formed by government agencies such as HHS and the USDA;
- increase oversight of laboratories (eg, survey number and capacity of laboratories, moratorium on new construction of laboratories, etc);
- increase oversight of laboratory personnel;
- standardize training of laboratory personnel; and
- improve system for reporting laboratory accidents.

The study cautions that, while additional oversight would be useful to monitor and promote facility and personnel licensure, personnel training, and restrictions on construction of new facilities, there would also be increased regulations and spending that could hinder scientific research.²

Analyses in the current study focus on differences between facilities with select agent or non-select agent status built during the period from 1990 to 2009. Should the number of BSL-3 facilities continue to grow, the burden of maintaining the structural safety of the facility as well as personnel training will increase.

The average cost of training a research scientist to work in a BSL-3 or BSL-4 is \$4,000 to \$7,000, and training support staff such as administrators can cost around \$4,000.²⁶ While most respondents in the current study did not allow support staff to enter the BSL-3, those that did had fewer training components for support compared to scientific staff. Support staff would likely require less specialized training than scientific staff, and the degree of training would vary among laboratories. Other studies have shown that BSL-3 facilities use more hands-on training

compared to BSL-2 facilities.¹⁷ Similar to our findings, lecture-based training was more common than hands-on training.¹⁷ In order to maximize retention, the type of training offered (hands-on versus lecture-based) should be customized based on the activity;¹⁷ it may be sufficient to use lecture-based in some training, but more useful to use hands-on in other training. Most select agent facilities included hands-on training, and not all facilities required written testing. Competency-based training and testing with continual mentoring from senior scientists²⁶ would assist trainees in learning microbiological practices. Despite the BMBL guidelines²¹ that workers must receive annual training updates, or additional training when policies or procedures change, 2 select agent facilities and 4 non-select agent facilities answered that they did not renew BSL-3 training. The lack of required follow-up training could allow changes in proficiency to go unnoticed or result in improper use of new equipment or facility features, which could jeopardize personnel safety and containment.

Respondents working in select agent laboratories relied on autoclaving and chemical decontamination more than non-select agent laboratories that used autoclaving. It is unknown whether this difference is due to the higher incidence of chemical decontamination of liquid waste, and this should be investigated further. The BMBL lists heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation as methods for sterilization.²¹ It is recommended that laboratories completely decontaminate their facilities after pathogen contamination. Most select agent and non-select agent facilities do not transport materials outside of the laboratory for decontamination. Differences in local and/or state waste regulations among facilities may account for some of the observed variation. This should be investigated further.

The current study showed that select agent facilities used disposable PPE more frequently than non-select agent facilities, and it is important that worker PPE (eg, gloves, gown, face and respiratory protection) be considered in biosafety risk assessments. Pathogens can survive on PPE and transfer to hands when gowns are being removed.²⁷⁻²⁹ We found that select agent laboratories primarily use PAPRs, while non-select agent laboratories use N95 respirators. This difference may be explained by the agents being handled and would be expected to vary among laboratories. While fit testing was performed annually at most select agent and non-select agent facilities, 8% of facilities performed fit testing less than annually or only as requested. Our study did not assess differences in respiratory protection based on specific agents. Organisms require the use of a BSL-3 laboratory, in part, because of infection risk due to aerosols. Although respiratory protection is not mandated, failure of engineering or equipment would make respiratory protection a last resort for worker protection. HEPA filters are not required in the exhaust of BSL-3 laboratories in all circumstances, and it is recommended that these filters be replaced as needed.

The BMBL recommends (but does not require) that facilities collect serum samples from employees working in BSL-3 laboratories.²¹ In the current study, medical surveillance was provided for scientific staff at all facilities, and most provided this service to support staff. However, $\leq 65\%$ of select agent and non-select agent facilities collect serum samples from scientific staff. These differences in surveillance among facilities could be due to relative differences in health risks between pathogens in different laboratories and this should be evaluated further.

Given the higher security required for select agents, it is expected that select agent facilities would use more restrictive access. We show that most select agent facilities used personalized methods (eg, personal access codes), indicating the importance of limiting entry to these laboratories. In contrast, general access methods were used more often in non-select agent compared to select agent facilities. The type of ongoing pathogen research in specific laboratories likely contributes to differences in access methods among facilities.

Most facilities answered that they received adequate funding. The lack of adequate funding could lead to a variety of safety issues regarding building and/or equipment maintenance or an increase in transport of materials out of the laboratory for decontamination or chemical decontamination methods, which could be due to broken equipment (eg, autoclave) or local laws. The current survey did not assess differences in local or state waste transport regulations that could have contributed to observed variation in decontamination procedures.

Pest management was carried out more commonly by select agent personnel compared to non-select agent personnel. This may be related to the increased restrictive access to select agent laboratories, making access by commercial pest management personnel more difficult. An integrated pest management program (including performing needed structural repairs to exclude pests) is recommended to prevent pests from contaminating and potentially mechanically transmitting pathogens.²¹

It is important that biosafety guidelines be followed and monitored primarily by fellow laboratory personnel and facility health and safety personnel to provide the greatest level of protection. Government agencies can be used for periodic reviews of laboratory procedures and could provide suggestions for improving SOPs. Implementation of programs similar to the personnel reliability program (PRP) employed by the National Biodefense Analysis and Countermeasures Center³⁰ could be useful tools if required by the select agent rules. The aforementioned PRP advocates creating a learning-based work environment, where reporting a laboratory mistake is encouraged. Since budgeting data were not received from all responding institutions, and funding deemed inadequate at one institution might be adequate at another institution, it was difficult to draw conclusions about what level of funding was inadequate.

The current study supports the need for each institution to use appropriate methods to provide the greatest level of safety for the staff and the public. Safety measures and training should be individualized for each laboratory; however, it may be useful for the government to provide suggestions to improve worker training and help laboratory managers identify and plan for long-term maintenance costs. Further studies are needed to address safety concerns for specific laboratories to determine the extent to which concerns are related to interactions between factors such as level of funding, pathogens used, and training methods.

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