

**Centers for Disease Control and Prevention
1600 Clifton Road
Atlanta, GA 30333
May 2, 2007**

Attention: 5th Edition BMBL Steering Committee

Dear Sirs,

The Technical and Regulatory Review Committee is part of the American Biological Safety Association (ABSA). ABSA is an international group of biological safety professionals which is known as one of the world's foremost resources on biological safety practices. We have reviewed the on-line version of the 5th Edition of the Biosafety in Microbiological and Biomedical Laboratories (BMBL). The new and revised sections should be of great value to practicing biosafety professionals. We have the following comments to share with you, and will share them section by section.

Section II: Risk Assessment (RA)

We commend the movement of this section to the front of the BMBL. Its movement recognizes the need to engage in the risk assessment process before one considers any other aspect of work with infectious agents.

Comment: The section indicates that the results of the RA should be used to alert staff to the hazards of working with the infectious agents and to the need for developing proficiency in the use of selected safe practices and containment equipment. It is suggested that you also consider specifically noting that work with toxigenic agents be included in this RA process, since there is now a separate section on Biological Toxins in the 5th edition of the BMBL. The risk assessment definition should be edited to note, that risk assessment is "the process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or toxigenic material."

The concept of Risk Groups is introduced for the first time in BMBL, and a table of the World Health Organization (WHO, 2004) and NIH (2002) definitions are given as defined by the (WHO). "They correlate with, but not do not equate to, biosafety levels. The risk assessment must still be done, to determine if the risk group will correlate with the biosafety level. (WHO) has recommended an agent risk group classification for laboratory use that describes four general risk groups based on the principal agent characteristics above and the route of transmission of the natural disease."

Comment: The WHO has recognized that the use of risk groups can be a systematic, effective way of considering multiple risks and hazards of infectious agents before engaging in their use. If they are to be noted within the BMBL, then it should be explicitly noted that the aerosol route of transmission must also be considered by anyone referencing the BMBL as part of the RA process. The WHO risk group process generally notes the need to consider route of transmission when assigning a risk group, but does not specifically reference aerosol route of transmission, which is a major consideration when establishing appropriate containment needed to handle infectious agents at BSL-3.

Comment: In this section, there are multiple references made to "infective dose." These references should be changed to "infectious dose" to be more consistent with terminology used within the BMBL and elsewhere. The updated BMBL also makes reference to the need to report laboratory acquired infections so that these events and their causes can be better tracked. If a specific mechanism has been established by the CDC for the reporting of such events, then it is best to reference it here (who should report, when reports are to be made, what is to be reported, how reports are to be filed, etc.)

The serious risk from agents that can be transmitted by the aerosol route is pointed out.

The transmissibility of an agent to a cagemate as an indicator of potential for aerosol transmission does not always hold true since some high risk agents (*Francisella tularensis*, *Coxiella burnetii*, and *Coccidioides immitis*) are not transmitted to cagemates.

Comment: The information that is used in this section regarding the transmissibility of these CDC Select Agents in cagemates is very useful information to anyone engaged in the RA process. In order to enhance accessibility of these facts to individuals engaged in the RA process who are preparing to work with these agents, it is recommended to move these references of cagemate transmissibility to the appropriate any agent summaries for these infectious agents that are in Appendix D.

Procedures that impart energy into a microbial suspension whether by hand or equipment create aerosols. The number of respirable size particles is outnumbered by the larger droplets that settle out on work surfaces and gloved hands. "The potential risk from exposure to droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols."

Comment: This statement regarding the consideration of surface contamination from droplets and aerosols as a RA consideration is commendable.

Section IV: Laboratory Biosafety Level Criteria

General Comments regarding Section IV and Section V: Increased stringency has been introduced into BSL-1 and BSL-2 procedures. Justification to support increasing the level of precautions for many of the practices has not been demonstrated in all cases; implementation of these changes may impose a significant burden on a number of institutions. This is especially true for work at BSL-1 infectious agents. These agents are well characterized which are not known to cause disease in healthy individuals. Further, there typically are a number of medical treatments available for individuals with personal risk factors that may experience a rare infection as a consequence of exposure to these agents.

There are a number of statements detailing personal protective equipment recommendations in these sections. There are also a number of statements within these sections where personal protective equipment (PPE) requirements are detailed. A number of these statements are inconsistent with one and other. We have sought to identify some of them in the text of these comments.

Posted signage should provide the critical information; anything else posted that detracts from that critical information can result in all warnings and cautions being ignored. Any information that is not critical at the moment of entry into the area, such as general occupational health requirements, should be included as part of the required biosafety manual.

Numerous changes are introduced without any apparent improvement in containment: these changes pose challenges for facilities that also must abide by the NIH Guidelines for Activities Involving Recombinant DNA Molecules since they would be inconsistent with these NIH guidelines. Specific examples include the new requirements for supervisors to enforce institutional policies regarding access to BSL-1 and BSL-2 laboratories.

BSL-1

Comment of A 5b: This statement should read," Used disposable needles and syringes must be carefully placed in conveniently located puncture-resistant and leak resistant containers for sharps disposal.

Comment on A.5.d: The recommendation that plasticware be eliminated at BSL-1 seems excessively precautionous. Furthermore, this revision introduces an inconsistency with the NIH Guidelines, as noted in A1, and A3. In the absence of evidence by the harm posed by glassware at BSL-1, the wording of the 4th edition should be retained for these provisions.

Comment on A9: The statement in indicating, "A sign incorporating the universal biohazard symbol must be posted at the entrance to the laboratory when infectious agents are present." In order to address this

requirement when infectious agents are present, many doors of BSL-1 laboratories would likely be posted in the manner described on an on-going basis, and not just when the infectious agents are present. This practice would diminish the significance of this posting as a means of communicating potential biohazards anywhere in the facility especially institutions which may also do work at higher biosafety levels in the same buildings. Regardless, for work with BSL-1 infectious agents, this posting provision should be removed for BSL-1 laboratories.

Comment on C.3: Eye protection should be worn whenever an individual is in a lab, no matter what the activity being conducted, because splashes are not always predictable.

Comment on C.4: Requiring gloves to be worn for protection from hazardous materials is inconsistent with the premise that agents causing human diseases are not handled under BSL-1 containment, if the hazardous materials referenced are the BSL-1 infectious agents. If the term hazardous materials are meant to be inclusive of such things as hazardous chemicals, and radioactive materials, then such a recommendation is warranted. If this is case, then it should be explicitly stated. Furthermore, this revision introduces an inconsistency with the NIH Guidelines. An apparent risk basis for this revision appears to be lacking.

Comment on D.4.b: Chairs with non-porous material should just be a recommendation at BSL-1, not a requirement. In the absence of evidence of harm posed by chairs covered with porous material, this requirement is not justified.

BSL-2

Emphasis on sharps precautions has been removed from the introductory paragraph to BSL-2 and it is not mentioned at all in BSL-1. Accidents involving sharps that lead to laboratory acquired infections are still major health hazards in a microbiology lab. The emphasis on these types of precautions gained by their reference in this paragraph should not be removed in these opening statements.

The need to place BSL-2 laboratories in non-public areas that was included in the 4th edition of the BMBL has been removed from this version. Since the BMBL may be referenced by biosafety professionals who are new to biosecurity issues, it would be good to make the implicit explicit here and retain the requirement.

Lab coats and eye protection should be required for all BSL-2 workers entering their BSL-2 laboratory. Splashes and spatters may occur when workers not working with the infectious agents are proximal to other workers who are performing this work. If lab coats and eye protection are worn by all of these workers, they are better protected against these incidental events, and they are better prepared to perform their own work with the infectious agent.

Comment of A 5b: See comment for A5.b. for BSL-1.

Comment on A.5.d: See comment for A.5.d. above for BSL-1.

Comment on A.9: The new version requires signage if an infectious agent is “present,” i.e., even if it is in storage. Agents in storage do not present a risk at BSL-2. The verbiage of the 4th edition should be retained (requires posting biohazard information if/when the infectious agent is in use). Also, signage at the entry to a lab is the appropriate place where individuals will seek instruction for entering the lab, but not for exiting.

Comment on B3: Medical surveillance may be appropriate for work with some BSL-2 infectious agents. (e.g., Hepatitis B Virus). Serum banking for all BSL-2 agents would not be universally warranted. Medical surveillance should be required as per the results of a risk assessment for the infectious agents in use and as appropriate in any infectious agent summary. Serum banking for specific at-risk personnel may be part of that medical surveillance effort, but it should not be a universal requirement for work at this level, since it may not be warranted.

Comment on B.9: Unless this new provision which disallows plants is based on evidence that their presence presents a risk, it should be deleted for BSL-2 agents.

Comment on B10: It is good that recognition is being given to the generation of possible aerosols at this biosafety level and the need to contain them.

Comment on C.3: Eye protection should be worn whenever an individual is in a lab, no matter what the activity being conducted, because splashes are not always predictable.

Comment on C.4: This statement requires gloves be worn as protection from hazardous materials. Instead, it should be based on a risk assessment and depend on activities to be conducted. If the term hazardous materials are meant to be inclusive of such things as hazardous chemicals, and radioactive materials, then such a recommendation is warranted. If this is case, then it should be explicitly stated. Furthermore, some of the agent summary statements are inconsistent with this statement by allowing discretion regarding the use of gloves (e.g., The *Listeria* statement says “gloves should be worn”). Also, alternatives to latex gloves should be available, as is noted in C4 of BSL-3.

Comment on D11: This statement indicates, “A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method).

This is a new recommendation for facilities do work at BSL-2. The text indicates that all laboratory wastes would need to be treated on-site, and not just laboratory wastes generated from the testing of infectious agents and discarded cultures and stocks of infectious agents. There is an absence of references in the scientific literature indicating occupationally acquired infections from medical waste since the last edition of the BMBL that would support this new requirement. Many hospitals have clinical laboratories working at BSL-2 within them, and many of these institutions would not have the means of treating all laboratory waste in this manner since many hospitals have discontinued incineration and other medical waste treatment over time. These institutions use licensed medical waste contractors to have their medical wastes treated off site. In absence of a technical reason for this requirement, and in light of the excessive burden and expense it would place on patient care, this provision should be dropped for BSL-2 facilities.

BSL-3

Comment on A.5.b: See A5.b for BSL-1 and BSL-2.

Comment on B.3: See Comment B3 above for BLS-2. Any serum banking should be rolled into the medical surveillance requirements for BSL-3 agents as determined by the risk assessment or detailed by the specific agent summary in the BMBL.

Comment on B.7a: A statement should precede this section stating, “BSL-3 facilities must develop plans for dealing with spills of infectious materials.” Knowledge of the number of room air changes per hour would be needed as part of this effort so that a determination can be made when it is safe for properly prepared personnel to re-enter the room to clean up the spilled material. Another statement should be added indicating, “BSL-3 facility staff needs to be made aware of the plan and how to initiate its provisions in the event of spills of infectious materials.”

Comment on C.3: Eye protection should be worn whenever an individual is in a BSL-3 laboratory, no matter what the activity being conducted, because splashes are not always predictable.

Comment on C.5: The determination about wearing a face shield and/or respirator should be based on the risk assessment, not an absolute requirement as written. There are circumstances where it is not necessary to wear a face shield.

Comment on D.12: The statement should read, “These HEPA filters should be tested at least annually and replaced as indicated by the trends in the HEPA filter testing.” HEPA filters in some biocontainment devices such as biological safety cabinets will last for years. Their removal yearly may secondarily loosen some connections within the biosafety needed to ensure containment. If the HEPA filter testing is done and is monitored for decreasing performance, then these filters can be changed in advance of failure without sacrificing the other containment benefits. HEPA filters in biocontainment devices that cannot be accessed for annual testing in this manner should be changed annually.

Table 1: Summary of Recommended Biosafety Levels for Infectious Agents

The summary tables for the Biosafety Levels were not updated to reflect changes to the content of each Biosafety Level 1-4. Once the content of each of the Biosafety Levels is finalized before printing of the document, this table needs to be reviewed and appropriately updated.

Table 1: Summary of Recommended Biosafety Levels for in Which Experimentally or Naturally Infected Vertebrate Animals Are Used

The summary tables for the Animal Biosafety Levels were not updated to reflect changes to the content of each Animal Biosafety Level 1-4. Once the content of each of the Animal Biosafety Levels is finalized before printing of the document, this table needs to be reviewed and appropriately updated. Also, the word, “Vertebrate” is misspelled in the Table description (corrected above). The Table number should also be changed from “1” to “2”.

Section V: Vertebrate Animal Biosafety Level Criteria, for Vivarium Research Facilities

General Comment: The introductory paragraph indicates situations for which this chapter is relevant: experimentally infected animals and lab animals that may harbor zoonotic infectious agents. Another situation should be added: immune compromised animals that receive grafts of cells derived from humans or Old World monkeys.

ABSL-1

General comments: Biohazard signage is not required for these agents, but it is earlier required for BSL-1 agents. In the standard practices section, the use of lab coats by workers is required at this animal biosafety level, but in the special practices section, use of lab coats is recommended. Also, the provisions regarding glove use in A7, standard practices, and in B4, special practices, are essentially identical. The glove provisions in the special practices section should be deleted.

Comment on A1: Requiring IBC review of all animal protocols is beyond the scope of an IBC. The animal protocol is a specific document that focuses on animal welfare and is the purview of the IACUC. Review by IBCs should be limited to specific activities involving infectious, toxigenic agents, allergenic agents and recombinant agents. (i.e., activities that may pose a health issue to the lab worker or environmental issue.)

Comment on A5: Signage needs to be minimal and to the point. To post all the information required by this section on a sign at the animal facility entrance results in a posting where the critical information will be ignored. As written (“and/or”), a sign must be posted everywhere animals are housed, without consideration of the presence of infectious agents, yet incorporating the biosafety level. There should be no biohazardous risk to humans at ABSL-1 and therefore no sign regarding the biosafety level. A different sign would be appropriate if containment is used for an animal pathogen or chemical hazard, and such posting should be associated with the specific room, not the entrance to the animal facility. Posting “general occupational health requirements” on a sign at the entrance to an animal area is not relevant for entry. General information belongs in the facility manual. Specific and unique occupation health requirements should be associated with specific animal housing rooms.

Comment on A7: The requirement to wear protective clothing is contradicted in C.2 (“recommended”). As written, gloves must always be worn when handling animals. This requirement is hard to justify based on biohazard at ABSL-1. Discretion should be allowed to use gloves as deemed necessary by the risk assessment. The similar statement for ABSL-1 under C.4 allows discretion (“should”) with regard to wearing gloves. The equivalent statement for ABSL-3 uses “should” allowing discretion.

Comment on D.1: There appears to be a typographical error in the statement, “Doors...animals are housed or open inward...” This same error appears in other sections.

ABSL-2

Comment on A1: Requiring IBC review of all animal protocols is beyond the scope of an IBC. The animal protocol is a specific document that focuses on animal welfare and is the purview of the IACUC. Review by IBCs should be limited to specific activities involving infectious, toxigenic agents, allergenic agents and recombinant agents. (i.e., activities that may pose a health issue to the lab worker or environmental issue.)

Comment on A2: This statement should read, “the safety manual should be prepared and (not “or”) adopted.”

Comment on A15: The statement should read “Decontaminate ~~of~~ all...”

Comment on B3: The recommendation to autoclave waste prior to incineration is excessive for ABSL-2. Proper waste treatment by either of these two methods should be acceptable.

Comment on D1: There is a typographical error: “Doors...animals are housed or open inward...”

Comment on D3: As stated, sealing penetrations is now required for all ABSL-2 facilities. This revision introduces a significant burden for institutions to implement. In the absence of evidence of harm from non-sealed facilities, this statement should be revised to be a recommendation, not a requirement.

ABSL-3

Comment on A1: Review by IBCs should be limited to specific activities involving infectious, toxigenic agents, allergenic agents and recombinant agents. (i.e., activities that may pose a health issue to the lab worker or environmental issue.)

Comment on A2: The statement should read, “The safety manual should be prepared and (not or) adopted.

Comment on A5: The signage requirement fails to address the complexity of some animal facilities, which are likely to have rooms at ABSL-1, -2, and -3. Specific information should be posted on the door to each animal room, not at the entrance to the animal area. Advance consideration of emergency and disaster recover plans does not fit the context of signage requirements; this information belongs in the facility operations manual.

Comment on C3: Section II of the BMBL indicates that a risk assessment should be undertaken before beginning to work with infectious agents. Use of respiratory protection should be based on the risk assessment.

Comment on C4: The statement regarding use of gloves for ABSL-3 allows more discretion than parallel statements for lower risk situations.

Section VI: Principles of Laboratory Biosecurity

In general, this section is well written and should be able to provide fundamental guidance in this topic for sites which work with infectious agents but which do not work with CDC or USDA Select Agents and Toxins. Some comments are provided below that may enhance its benefit. These comments are provided since this section provides much greater beneficial guidance than in the 4th edition of the BMBL, and some newer biosafety professionals may not have previously dealt with biosecurity issues.

The utility of this section in providing this guidance could be helped if there is some discussion of the sections of the Select Agents and Toxin requirements which were considered in the writing of the text of this section, and how the security requirements detailed in this section may have some overlap with the requirements of other agencies and groups which deal with security issues. References to the following documents in addition to the ones already cited may prove useful to the reader:

1. USDA-ARS DM 9610-001, Departmental Manual "USDA Security Policies and Procedures for Biosafety Level-3 Facilities, August 30, 2002
2. USDA-ARS DM 9610-2, Departmental Manual "Security Policies and Procedures for USDA Laboratories and Technical Facilities Excluding BSL-3 Facilities", April 30, 2003
3. DoD Instruction 5210.89, "Minimum Security Standards for Safeguarding Biological Select Agents and Toxins", April 18, 2006
4. DoD Directive 5210.88, "Safeguarding Biological Select Agents and Toxins", February 11, 2004
5. 32 CFR Part 626, "Biological Defense Safety Program", July 1, 2006
6. 32 CFR Part 627, "The Biological Defense Safety Program, Technical Safety Requirements (DA Pamphlet 385-69)", July 1, 2006
7. World Health Organization's, "Biorisk management: Laboratory biosecurity guidance", Document WHO/CDS/EPR/2006.6, September 2006

It should be noted that biosecurity requirements for dual use may make it imperative that the risk assessment portion of this chapter incorporates a mechanism to identify experiments that may require compliance to dual use requirements once they are established and finalized by the National Security Advisory Board for Biosecurity (NSABB). Provision of a web link to the NSABB web site would assist dual user readers of the BMBL in effectively investigating this aspect of their risk assessment:
<http://www.biosecurityboard.gov/>

The institutional review of research and the dissemination of the concept that certain technologies and products should be incorporated in this chapter as a corollary to physical security and the need for operational control of agents because in dual use, the experimental design and technology that can yield harmful results must be secured every bit as much as the actual agent so perhaps an expansion to include IT security. The dissemination of publications and abstracts may also be added to this chapter.

Lastly, it may be useful to mention the need for government security clearances and the process; the option of biological surety and reliability programs similar to chemical and nuclear surety program is another potential option in the design of a biosecurity program. This article summarizes this concept that includes the concept of personnel reliability screening which may also be pertinent in this chapter.

<http://www.liebertonline.com/doi/abs/10.1089/153871304322964291>

Section VII: Occupational Health and Immunoprophylaxis

There is a sentence in the fifth paragraph that should be changed to, "Medical support services for biomedical research facilities should be evaluated annually or whenever there is a change in potential risk."

Occupational Health Support Services

Pre-placement Medical Examinations

There is a statement, "The health provider should review the worker's previous and ongoing medical problems, current medications, allergies to medicines, animals and other environmental proteins, and...."

Comment: This statement should read, “The health provider should review the worker’s previous and ongoing medical problems, current medications, allergies to medicines, animals and other environmental allergens, and....”

Vaccines

The last sentence of the third paragraph states, “Receipt of such vaccines is rarely justified as a job requirement.” This statement is an opinion which may or may be applicable in any given situation. Since it is an opinion and not specific guidance, the need for this statement is questionable, and its removal should be considered.

Medical Support for Occupational Illness and Injuries

The fifth paragraph discusses post-exposure prophylaxis. It is suggested to add the sentence, “If the potential for any post-exposure prophylaxis exists, the worker should be fully informed prior to beginning the work and a course of action determined for each worker taking into account the possibilities of such issues as pregnancy, co-morbid disease state, a significant immunocompromised state, etc.” This pre-defined course of action is especially important if the exposed person is unable to communicate to medical responders at the time of incident.

The list of items that the medical provider’s description of injuries should include in this section should also include the following items:

- The possible amount of the potentially infectious agent received by the patient
- The task being undertaken when the incident occurred.

Occupational Health in the BSL-4 Setting

The fifth sentence states, “Thus, SOPs for BSL-4 settings require special attention to management of unexplained worker absence, including protocols for monitoring, medical evaluation, work-up, and follow-up of workers with unexplained absences.” These SOP’s should be required for BSL-3 settings as well. Both BSL-3 and BSL-4 facilities would be working with infectious agents which are transmissible via the aerosol route. Infection from these agents via this route would therefore not always be apparent. Some of these infectious agents may have not have unique signs and symptoms which could be readily recognized and tracked, which places a greater reliance of more passive surveillance via the general indicators of illness noted in this statement.

Section VIII: Agent Summary Statements

Section VIII-A Bacterial Agents

Mycobacterium Tuberculosis Complex Agent Summary

There is a statement, “BSL-3 practices, containment equipment, and facilities are required for laboratory activities in the propagation and manipulation of cultures of any of the subspecies of the *m. tuberculosis* complex and for animal studies using experimentally or naturally infected NHP.....BSL-3 practices should include the use of respiratory protection and the implementation of specific equipment to prevent and contain aerosols.”

Comment: The second half of this statement could infer that respiratory protection should be used anytime that aerosol generating procedures are being conducted. If these procedures are being conducted in a BSL-3 facility in a certified biological safety cabinet by a worker trained in the proper and safe use of a biological safety cabinet who is using this training, then respiratory protection should not be necessary. There is no similar wording regarding possible additional respiratory protection in the other agent summaries for infectious agents that are to be used in BSL-3 facilities. It is suggested that the

second half of the statement be re-worded to state, “BSL-3 practices should include the use of respiratory protection whenever aerosol generating procedures are conducted outside of a biological safety cabinet.”

Special Issues

There is a statement, “Annual or semiannual skin testing with purified protein derivative (PPD) of previously skin-test negative personal can be used as a surveillance procedure.”

Comment: Volume 54, RR-18, “Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings 2005”, was published by Morbidity and Mortality Monthly Reports (MMWR) on December 30, 2005. As per that updated document, the term tuberculin skin testing (TST) has been adopted in place of the term “PPD”. In addition, the frequency of TB medical surveillance is to be determined through a risk assessment that considers the incidence of TB disease in the community in which the health care facility is based and the incidence of TB within the health care facility. Guidance on medical surveillance should reference these CDC recommendations. It would be helpful to include a statement that the QuantiFERON[®] assay has been approved by the Food and Drug Administration for use in TB medical surveillance. Knowledge of the availability of this assay may help investigators with medical surveillance issues and could greatly assist in post exposure medical evaluations should they be necessary.

References

Comment: This agent summary cites a reference for the statement, “Animal studies using guinea pigs or mice can be conducted at ABSL-2.” The reference noted does not discuss animal studies. The reference cited discusses how to set up a clinical laboratory for TB analysis. The reference should be deleted and the appropriate reference for animal studies inserted, once it can be confirmed. The reference that should be deleted for this statement is: 111. Richmond, et al. Biosafety in the Clinical Mycobacteriology Laboratory. *Clin. Lab. Med.*, 1996: 527-550.

Francisella tularensis Agent Summary

There should be a reference to the IND vaccine that is available, similar to the vaccine statement made for *bacillus anthracis*. Since this agent has a very low infective dose and caused many LAIs.

Section VIII-F—Viral Agents

Influenza

There is a statement, “Select Agent Influenza virus is a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information. Additionally, there is a statement,“ Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Comment: The requirements referenced above are for “highly contagious avian influenza”. Your agent summary should note “*highly pathogenic avian influenza*” and “*the 1918 influenza strain*,” only, when referencing these requirements so readers will not think that these Select Agent requirements apply to other influenza virus strains.

Table 1: Alphabetical Listing of 597 Arboviruses and Hemorrhagic Fever Viruses (Arboviruses)

Kunjin Virus

Comment: The recommended biosafety level for Kunjin virus is now listed at BSL-3 where it had been BSL-2 in the previous version of the BMBL. The basis of this rating is S, which appears to be a survey by

SALS. This is also in the reference given in the 4th edition of the BMBL when it was assigned BSL-2 level. The 1980 SALS publication lists Kunjin virus, and it is listed in that publication at BSL-2. If this is a change in BSL-3 levels, then a new reference should be noted that provides the technical reasons for this change. Otherwise, BSL-2 should be noted for this infectious agent.

Human Herpesvirus Agent Summary

It is stated in this agent summary that the agent summary for herpes virus simiae (B virus, Monkey B virus) is discussed separately. The agent summary for herpes virus simiae (B virus, Monkey B virus) has not been included in the 5th edition of the BMBL.

Newcastle Disease Virus (NDV) Agent Summary

There is a statement indicating that NDV “should be handled in vitro in a BSL-3 laboratory with enhancements as required by the USDA and in vivo in the USDA-approved BSL-3-Ag animal facility.”

Comment: NDV should be handled in vitro in a BSL-3 laboratory with enhancements as required by the USDA, in vivo in an ABSL-3 vivarium with enhancements as required by the USDA for small animals in animal enclosures, and in vivo in USDA-approved BSL-3-Ag animal facility for animals loose housed where the walls are primary containment.

Rift Valley Fever Agent Summary

Comment: There is a statement indicating that, “the USDA may require full BSL-3Ag containment”. Several lines later under the Special Issue heading, there is a statement indicating “the USDA may require enhanced ABSL-3 or ABSL-3 facilities and practices when working with RVFV in the US.” The intent of these two statements is unclear. The authors should reexamine them and clarify them for consistency.

SARS coronavirus Agent Summary

“Institutions performing work with SARS coronavirus should require storage of a baseline serum sample from individuals who work with the virus or virus-containing specimens.”

Comment: The best use of resources is to do what is described in the BSL-3 special practices section ... *Each institution must establish policies and procedures describing the collection and storage of serum samples from at-risk personnel.* The risk for laboratory acquired infection while working with SARS coronavirus is less than many other risk group 3 agents.

Appendix B: Decontamination and Disinfection

Environmentally Mediated Infection Transmission

Labs do not usually use chemical sterilants to clean surfaces. What are used are disinfectants or decontaminants. Sterilization is generally only done for wastes.

Principles of Sterilization and Disinfection

This section defines disinfection and sterilization but not cleaning, germicide, or sanitization.

Sterilization

Regarding “sterility assurance level,” This could be interpreted that only 1 in 1 million is a sterility assurance level which would be inaccurate. It would be best to add that “the sterility assurance level of a sterilizing process is the degree of assurance with which the process renders a population of items sterile.” Quoted from Disinfection, Sterilization, and Preservation. 5th Ed. Seymour Block (Ed.), Chap. 70, p. 1361.

Disinfection

The statement is made, “Disinfection is a procedure that reduces the level of microbial contamination, but there is a broad range of activity that extends from sterility at one extreme to a minimal reduction in the number of microbial contaminants at the other.”

Comment: Disinfection is a procedure that reduces the level of pathogenic contamination. Decontamination is the procedure that reduces the level of microbial contamination.

The statement is made, “Some germicides rapidly kill only the ordinary vegetative forms of bacteria such as staphylococci and streptococci, some forms of fungi, and lipid containing viruses, whereas others are effective against such relatively resistant organisms as *Mycobacterium tuberculosis* var. *bovis*, non-lipid viruses, and most forms of fungi.”

Comment: The term, “germicide” is never defined in this appendix. In this case the author is using germicide to be synonymous with disinfectant or decontaminant.

High-Level Disinfection

The statement is made, “These chemical germicides are potent sporicides and, in the United States, are classified by the FDA as sterilant/disinfectants. They are formulated for use on medical devices, but not on environmental surfaces such as laboratory benches or floors.”

Comment: The FDA registers those disinfectants intended for use on medical devices only. High level disinfectants are used on environmental surfaces routinely in the biotechnology and pharmaceutical industries.

Decontamination and Cleaning

The statement is made, “If dangerous and highly infectious agents are present in the laboratory, the methods for decontamination of spills, laboratory equipment, BSC, or infectious waste are very significant and may include prolonged autoclave cycles, incineration or gaseous treatment of surfaces (see below).”

Comment: Decontamination is always very significant. Does the author mean that in this case it is significantly different than when working with Risk Group 2 organisms? Even with Risk Group 2 organisms, surfaces are generally treated with disinfectants and not gaseous (except for pre-maintenance or general shut-down). If this is in reference to Risk Group 4 organisms only, then that should be made clear.

Table I: Descending Order of Resistance to Germicidal Chemicals

This table should be updated to note the following descending order: prions>bacterial spores>coccidia>mycobacteria, and then the rest of the table remains the same.

Decontamination of Large Spaces

The statement is made, “Thus, in the BSL-3 laboratory, surface decontamination, not fumigation, is the primary means of decontaminating space.”

Comment: It should be made clear here that in many BSL-3 facilities, fumigation is done on a periodic basis, such as when certain maintenance activities are conducted or sometimes when the infectious agent in use in the laboratory changes.

The statement is made, “Verification of seals is usually not required for most BSL-3 laboratories.”

Comment: This step is required for ABSL-3 facilities. Also, in the National Institutes of Health Design Criteria for BSL-3: "D.8.2.8 Penetrations and Joints: All penetrations in walls, floors, and ceilings shall be sealed with a smooth finish to facilitate decontamination and cleaning. All joints between fixed cabinetry and equipment (e.g., shelves, cabinets, plumbing fixtures, etc.) and the floor or wall shall be smooth covered and sealed to ensure maximum cleanability. Supply and exhaust ducts shall be gasketed or sealed at the point of penetration into the laboratory to ensure containment and the capability of gas decontamination. Light fixtures in BSL-3 laboratories shall be surface or pendent mounted."

Most BSL-3 labs designed today are capable of being pressure tested, which means verification of the seals as part of the facility certification process. This is particularly the case in NIH funded laboratories (RBLS). Since these labs are generally subjected to gaseous decontaminations, verification of seals must be in place prior to fumigation.

Formaldehyde-paraformaldehyde

The statement is made, "Formaldehyde gas at a concentration of 0.3 grams/cubic foot for four hours is often used for space decontamination.

Comment: This concentration is less time than the National Sanitation Foundation recommends for a BSC. It is recommended changing this statement to read: "...0.3 grams/cubic foot for a minimum of six hours...."

The web link, www.epa.gov/pesticides is referenced in this section. It is recommended that guidance be given by the following alternative web link: www.epa.gov/pesticides/factsheets/paraformaldehyde_factsheet.htm, which will allow direct access to the paraformaldehyde fact sheet.

Hydrogen Peroxide Vapor

The statement is made, "Hydrogen peroxide can be vaporized and used for the decontamination of glove boxes as well as small room areas. Vapor phase hydrogen peroxide has been **shown to** be an effective sporicide at concentrations ranging from 0.5 mg/L to <10 mg/L."

Comment: Vaporized hydrogen peroxide has also been for decontamination of large spaces.

Chlorine Dioxide Gas

The statement is made, "Chlorine dioxide possesses the bactericidal, virucidal and sporicidal properties of chlorine, but unlike chlorine, does not lead to the formation of trihalomethanes or combine with ammonia to form chlorinated organic products (chloramines).

Comment: It is unclear if the author is referencing chlorine gas or if they mean to reference sodium hypochlorite. While trihalomethane generation poses health hazards which should be avoided, the generation of chloramines does not pose comparable health hazards. Chloramines are used in sanitizers and disinfectants.

The statement is made, "Because chlorine dioxide gas exits the generator at a modest positive pressure and flow rate, the enclosure also need not be evacuated and could be a sterility-testing isolator, a glove box or sealed BSC, or even a small room that could be sealed to prevent gas egress. Chlorine dioxide gas is rapidly broken down by light; care must be taken to eliminate light sources in spaces to be decontaminated."

Comment: Since chlorine dioxide was used to decontaminate very large government buildings, I would remove the words, "even a small room."

Table II: Activity Levels of Select Liquid Germicides

The table notes that the aqueous concentrations of glutaraldehyde, chlorine dioxide, and peracetic acid are “variable” when used as sterilizing agents and disinfectants.

Comment: The EPA registered high level/sterilants are in a narrow band of 2.0-3.4% for glutaraldehyde. This level is 2% for chlorine dioxide. There are no strictly peracetic acid agents registered—only mixtures.

The table notes that the aqueous concentrations of iodophors are “30-50 mg/L free iodine up to 10,000 mg/L available iodine and 0.1-0.2%) variable” when used as disinfectants.

Comment: The concentration noted (0.1-0.2%) should be moved one line as this is the concentration for quaternary ammonium compounds not iodophors.

Footnote “c” of this table states, “Although the indicated concentrations are rapid acting and broad-spectrum (tuberculocidal, bactericidal, fungicidal, and virucidal), no proprietary hypochlorite formulations are formally registered with EPA or cleared by FDA.”

Comment: The EPA has over 3 dozen sodium hypochlorite disinfectants registered in their various lists.

Comment: It should be noted that Table 2 is a direct copy of Table 43.4 from ref. 6.

Special Infectious Agent Issues

Transmissible Spongiform Encephalopathy Agents (Prions)

The first paragraph in this section reads, “The major exception to the rule is the previous discussion of microbial inactivation and decontamination is the causative agent of CJD or other prion agents responsible for transmissible spongiform encephalopathies of the central nervous system in humans or animals. Studies show that prions are resistant to conventional uses of heat and/or chemical germicides for the sterilization of instruments and devices (see Chapter 9).

Comment: There is no Chapter 9 in the 5th edition of the BMBL; “Prion Diseases” are covered in section VIII-H of the 5th edition of the BMBL.

References

7. Centers for Disease Control and Prevention [www.cdc.gov]. Atlanta: The Centers for Disease Control and Prevention; [updated 2006 Sept 21]. Guidelines for Environmental Infection Control in Health-Care Facilities, 2003; [about 2 screens]. Available from: <http://www.cdc.gov/ncidod/hip/enviro/guide.htm>

Comment: This web site reference is out of date. Currently, it is http://www.cdc.gov/ncidod/dhqp/gl_enviroinfection.html

Appendix C :Transportation of Infectious Substances

Transportation Regulations

The United States has taken great strides over the past several years to harmonize its infectious substances and diagnostic/clinical specimen shipping requirements with the rest of the world. This effort has largely been concluded at the time that the 5th edition of the BMBL has been made available for review, and you have done well with managing this topic. Of practical value to the readers who may not be fully versed with these applicable shipping requirements for these hazardous materials is the fact that individual International Air Transport Association (IATA) members may have requirements that need to be addressed that may be more stringent than the requirements noted in the IATA Dangerous Goods Regulations. Shippers of infectious and diagnostic/clinical specimens would be well advised to consult

these regulations before bringing a shipment to an IATA carrier if rejection of the shipment is to be avoided by the IATA carrier.

It would also be helpful to provide the specific reference to the hazardous material security planning requirement in the list of regulations and to note the “security plan” and the “hazardous material security plan” referenced in those standards. These clarifications would provide better linkage of the regulation and better prepare shippers to address these aspects in their site’s security planning efforts. There could be some confusion if a shipper of Select Agent mistook the security plan that is required under the CDC Select Agent and Toxin as fully addressing all of the provisions of the U.S. Department of Transportation’s hazardous material security plan requirements. The CDC Select Agent security plan requirement is focused on the security of the Select Agent at the facility whereas the U.S. Department of Transportation’s security plan requirements are focused on security of the Select Agent while in transit to or from the Select Agent facility. The specific U.S. Department of Transportation standard requiring the preparation of these security plans is: 49 CFR Subpart I, 172.800, Security Plans.

General DOT Packaging Requirements for Transport of Infectious Substances by Aircraft

Comment: It would be helpful to indicate that the required emergency response information on the Shipper’s Declaration of Dangerous Goods must include an emergency phone number staffed 24/7/365 by someone knowledgeable about the hazardous materials being shipped in the package.

Comment: The reference to “Biological Specimen, Category B” is incorrect. The correct reference is Biological Substances, Category B. This reference should be corrected within the text of this section and in Diagram 2 of this appendix.

Although the references to Category A Infectious Substances and Biological Substances, Category B specimen provides useful information, the reality is that due to the time sensitive nature of many of these shipments, they are shipped via aircraft, and most common air carriers and commercial airlines are IATA members. IATA members require shipments which they are to carry to be prepared as per the Dangerous Goods Regulations of the IATA. It would be practical and beneficial to note this and to reference the IATA packaging instructions 602 and 650 in this section. It would be good to show examples of packaging and shipping of these hazardous materials as per these packaging instructions versus the U.S. DOT references.

Appendix D: Animal Pathogen Biosafety

Comment: The second sentence at this beginning of this section notes that all questions regarding its contents should be directed to the USDA. The means of contacting appropriate staff within the USDA for fielding such questions should be noted, or you should consider dropping this sentence.

II. BSL-3-AG for Work with Loose-Housed Animals

In item #4, there is a statement, “Disposable materials must be autoclaved before leaving the BSL-3-Ag space, and then incinerated.”

Comment: This statement should be written to state, “Disposable materials must be autoclaved before leaving the BSL-3-Ag space, and then appropriately disposed of according to state, or local laws.”

In item #5, there is a statement, “The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds appears when the preset pressure differential is not maintained.

Comment: This statement should be written to state, “The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds and visual display appears when the preset pressure differential is not maintained.” It is very hard to hear alarms while wearing powered air purifying respirators; a flashing light for an alarm is also recommended for inclusion.

In item #6, there is a statement, “The most severe requirements for these modern, high level biocontainment facilities include HEPA filters arranged both in series and in parallel on the exhaust side, and parallel HEPA filters on the supply side of the HVAC systems serving “high risk” areas where large amounts of aerosols containing BSL-3- Ag agents could be expected (e.g., animal rooms, contaminated corridors, necropsy areas, carcass disposal facilities, etc.).

Comment: This statement should be written to state, “The most protective requirements for these modern, high level biocontainment facilities include HEPA filters arranged both in series and in parallel on the exhaust side, and parallel HEPA filters on the supply side of the HVAC systems serving “high risk” areas where large amounts of aerosols containing BSL-3-Ag agents could be expected (e.g., animal rooms, contaminated corridors, necropsy areas, carcass disposal facilities, etc.).

There is also no reference for the need for emergency generators or other back power supply for these facilities. At minimum, there should be an emergency power supply to both the supply and exhaust fans, since these facilities are supposed to be air-tight, and these ducts could collapse if both systems are not continually powered. Consideration should also be given to provide emergency power to other emergency components such as exit lights and emergency lights.

In item #12, there is a statement, “Restraining devices shall be provided in large animal rooms.”

Comment: This statement should be written to state, “Physical restraining devices shall be provided to be used only when chemical restraint is not possible in large animal rooms.” It is very dangerous for staff to work with risk group 3 infected unanaesthetized animals in a large animal facility. The use of physical restraints should only be done as a last resort when no other option is available in order to more effectively manage the risks from these animals.

III. BSL-3 AND ABSL-3 + Potential Facility Enhancements for Agricultural Facility Permitting

In item #1, there is a statement, “Complete laboratory clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is provided in the “dirty” change room, and put on by personnel before entering the research areas.

Comment: This statement should be rewritten to state, “Complete laboratory clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is provided in the “clean” change room, and put on by personnel before entering the research areas.

In item #4, there is a statement, “Wastes and other materials being removed from the BSL-3 enhanced space must be disposed of through incineration or other approved process.”

Comment: This statement should be rewritten to state, “Wastes and other materials being removed from the BSL-3 enhanced space must be disposed of through incineration or other approved process meeting state or local requirements.”

In item #5, there is a statement, “The pressure differential display/gauge can be seen inside and outside of the containment space and an alarm sounds when the preset pressure differential is not maintained.

Comment: This statement should be rewritten to state, “The pressure differential display/gauge can be seen inside and outside of the containment space, and an alarm sounds and visual display appears when the preset pressure differential is not maintained.”

In item #6, there is a statement, “Air handling systems must provide 100% outside conditioned air to the containment spaces.”

Comment: There is also no reference for the need for emergency generators or other back power supply for these facilities. At minimum, there should be an emergency power supply to both the supply and

exhaust fans, since these facilities are supposed to be air-tight, and these ducts could collapse if both systems are not continually powered. Consideration should also be given to other emergency components such as exit lights and emergency lights.

IV. Pathogens of Veterinary Significance

There are 11 viruses detailed in the table with subscript “c” which is defined in the notes which follow it as, “requires BSL-3 Ag containment for all work with the agent in loose housed animals.” Section V, Summaries of Selected Agricultural Agents and the agent summaries of 10 of the 11 viruses (Rift Valley Fever Virus is in the arbovirus and is not in the Ag chapter.) indicate that these organisms should only be handled, “in vivo in a USDA-approved BSL-3-Ag facility” without any mention of ABSL-3 plus enhancements for small animals in isolators. Any of the agent summaries of the viruses with subscript “c” should be changed to make all of these sections consistent.

Appendix F: Select Agents and Toxins

There are no specific comments regarding the text of this section. We do wish to provide the comments below that were made following the review of the Section VI: Principles of Laboratory Biosecurity. These comments may be of value to readers of this section as they seek to develop biosecurity programs that deal with the CDC Select Agent and Toxin program requirements.

Biosafety and Biosecurity

In the paragraph on personnel qualifications, no mention is made of performing background checks on all staff that have or control access to the biological materials. The “insider” threat is alluded to under Step 2 of the Example Guidance but mention of it when referring to personnel qualification is appropriate and completes the picture of personnel assessment. Mitigating the internal threat to organizations from disgruntled employees or those who have been convinced to compromise their positions should be included as an element of a biosecurity plan. Recommending a security evaluation of staff may prove quite helpful for readers who have not faced this issue before. Organizations that already include a security evaluation may want to tailor the review to meet biosecurity concerns. The components to recommend for review may include: criminal activity, misuse of illegal or legal drugs, mental or emotional instabilities, financial vulnerabilities, etc. A personnel screening program is described in the MMWR report included as reference 1. Engendering the laboratory culture into staff may be facilitated by the use of a personnel reliability or “honor” program where each employee is responsible for biosecurity oversight.

The paragraph addressing personnel qualifications also addresses shipping, access, etc. Splitting the topic of personnel qualifications to its own paragraph may be useful for clarifying the topics to the reader. However, the identity of the personnel gaining access or shipping the materials is an important detail to track.

In the last sentence in this section and elsewhere the importance of biosafety to staff and the environment is appropriately listed as taking precedence over biosecurity. However, theft and misuse of the biological materials is a safety concern as well so the ability to recognize and report a theft quickly, to establish mechanisms for promptly making such reports, and to identify the pathogen(s) taken for the appropriate emergency responses to be mounted is a good mitigating response to having to choose between safety and security.

Risk Management and Methodology

The statement “Not all institutions will rank the same agent at the same risk level” is true; however, it may be good at this point to encourage institutions to base the risk assessment on peer-reviewed or well-researched evaluations. Institutes that find the risk tolerance is too high should not engage in the proposed work involving those high risk pathogens. This may be obvious but stating this clearly may have merit.

Developing a Biosecurity Program

It is important in the development of the biosecurity program that the accepted security measures do not introduce or reduce the mitigation of biosafety hazards. Therefore the following edit is recommended for the last sentence in the first paragraph in this section: *“This coordinated approach is critical in ensuring that the biosecurity program provides reasonable, timely, and cost effective solutions addressing the identified security risks without introducing any safety hazards, removing or compromising any safety measures, or unduly affecting the scientific or business enterprise....”*

The last paragraph in this section would be a good place to reference the guidance documents such as #7 referenced above for use in developing the biorisk assessment.

Example Guidance: A Biosecurity Risk Assessment and Management Process

The first paragraph that states “Different models exist regarding biosecurity risk assessment” is also a prime location to cite additional guidance documents such as reference #7.

Step 1: Identify and Prioritize Biological Materials

The last paragraph under Step 1 advises that no further action may be required should the biological materials be deemed not to pose a security risk above what is already accommodated for by the institution’s current policy. However, if misuse of the materials would pose a security risk, then the elements that should be evaluated that are mentioned in this section may be missed in this example. The items that may require further evaluation and potential mitigation include the physical vulnerabilities of location and access.

Step 2: Identify and Prioritize the Threat to Biological Materials

It is worthwhile to point out here that disgruntled employees likely pose the greatest “insider” risk. One step to help dilute this risk is to avoid placing all points of access control or physical access to biological materials in any single individual.

Step 3: Analyze the Risk of Specific Security Scenarios

In addition to identifying physical vulnerabilities, it is advisable to examine vulnerabilities in infrastructure, laboratory operations, and hiring practices.

Step 4: Develop and Overall Risk Management Program

Among the mitigation steps that management can take are those that screen job applicants, provide continuous evaluation of current employees, employ fair termination practices, and offering services to personnel that ultimately exhibit care for the well-being of staff.

Program Management

Excellent points are made in this section. The absence of management support for biosecurity or biosafety cripples the entire program.

Physical Security—Access Control and Monitoring

Defining and appropriately restricting who would have access to the physical security barriers is an important element of the program as well as widely communicating these restrictions to all who have access to the information.

Personnel Management

A laboratory staff “honor” or reliability program is an important element in managing staff. Having anonymity for any whistle-blowers is an important feature so that junior staff will have no repercussions for reporting senior staff. Staff that monitor themselves and contribute to laboratory operations policies can provide the best protection against adverse events.

Inventory and Accountability

It is recommended to add, “Determining how to manage, restrict, and track physical access to biological materials” to the list of items for management to define.

Information Security

This section includes some of the items listed above. Information that may also be considered sensitive includes the list of individuals who have or control access to the biological materials.

Transport of Biological Agents

Tracking is such an integral element of the select agent program that mentioning that here would be instructive and prudent for the reader to realize. Adding the sentence at the end, “The CDC and USDA select agent programs provide many details that can be considered for inclusion in any tracking program.” At minimum, the references to these programs could be cited at the mention of “regulatory” procedures in the last sentence in addition to referring the reader to Appendix C for shipping information.

Accident, Injury, and Incident Response Plans

It is recommend adding as the third sentence in this paragraph. “In addition, laboratory staff with access may be trained to serve as emergency responders in select and well-defined situations.”

Reporting and Communication

An item that is sometimes missed in laboratory designs is adequate communication from and within the laboratories for safety and security concerns. It is important to ensure that the location of potential incidents have communication means readily available to immediately report the occurrence of adverse events to the appropriate staff.

Training and Practice Drills

Mentioning that some regulations may define the minimum periodicity required for drills would be useful for those readers entering the field.

Security Updates and Reevaluations

It is recommend adding as the last sentence in this section, “Restricted and defined access to these records should be established in view of protecting vulnerability information for the organization.”

Appendix I: Guidelines for Working with Toxins of Biological Origin

Low Molecular Weight Toxins

Comment: Not all low molecular weight toxins are select agents.

Comment: The reference to “protective masks” for protection against possible aerosol generation needs to be clarified. Not all protective masks will provide proper and effective protection against respirable airborne biological toxins. Specific types of respirators which may afford proper protection should be noted in this statement.

Appendix J: NIH Oversight of Research Involving Recombinant Biosafety Issues

Comment: OBA is used as an abbreviation without stating what it is abbreviating. When Office of Biotechnology Activities is first used in this section, then follow it with (OBA), and then use OBA throughout the rest of this Appendix. (OBA is included in the list of abbreviations for this appendix.)

Due to the extensive nature of the revisions and additions to the updated BMBL, we have focused our review on the new or updated sections of the document. Please do not consider these comments to be a comprehensive review of all parts of this document. We would welcome more time to review the other sections prior to finalizing this document in hard copy. We recognize that this document is an invaluable reference used by many biosafety professionals here in the United States and in other parts of the world, and we appreciate the opportunity to have shared these comments with your organization.

**Bill Homovec, MPH, CBSP
Chair and Co Team Leader
ABSA Technical and Regulatory
Review Committee**