

# Summary of Changes to the Biosafety in Microbiological and Biomedical Laboratories 6th Edition (BMBL-6)

[CDC/NIH Biosafety in Microbiological and Biomedical Laboratories 6th Edition \(BMBL-6\) 2020](#)

[CDC/NIH Biosafety in Microbiological and Biomedical Laboratories 5th Edition \(BMBL-5\) 2009](#)

This Summary of Changes was prepared by the ABSA International Technical and Regulatory Review Committee (TRR).

**This Summary of Changes is not to be used as a substitute for the [CDC/NIH BMBL-6 edition](#).**

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## **Section I – Introduction (pages 1-7)**

The introduction provides inter-edition differences including those found in the Risk Assessment, Agent Summary Statements and Laboratory Biosecurity sections. One distinction that is clearly made in the Introduction is the idea that the BMBL 6th (BMBL-6) is not the only source of biosafety information. Stakeholders including the IBC, Biosafety Officers and others versed in biosafety concepts are included as participants when developing and performing a risk assessment.

## **Section II Biological Risk Assessment (pages 9-20)**

All areas covered under Section II Biological Risk Assessment in both the BMBL-5 and BMBL-6 are similar. In BMBL-6, the risk assessment includes a more comprehensive detailed approach. The risk assessment is outlined in a six -step approach that provides structure to the risk management process and reinforces an ongoing positive culture of safety. This follows the **PLAN, DO, CHECK, ACT** principle. The role of the risk assessment is described as part of an ongoing risk management process and is both stressed and linked to the concept of fostering a positive safety culture. This section also emphasizes the need for the inclusion of a broad range of stakeholders and identifies an expanded list of stakeholders including institutional leadership and Biosafety Professionals.

## **Section III—Principles of Biosafety (pages 24-31)**

Section III provides a brief introduction to biosafety. It also acts as a primer to Section IV. The BMBL-6 is very similar to the BMBL-5 in content and layout. Headings within this section include Facility Practices and Procedures, Safety Equipment (Primary Barriers), Personal Protective Equipment, Facility Design and

Construction (Secondary Barriers), Biosafety Levels, Animal facilities, Clinical Laboratories and Laboratory Biosecurity. The Laboratory Biosecurity header replaces the Select Agent header from BMBL-5 though the content is rather similar with a few additions reflecting current requirements. Lastly, the header entitled, Importation and Interstate Shipment of Certain Biological Materials, from the BMBL-5 has been completely removed though the information in this header can be found in Appendix C. Increased emphasis is placed on the hierarchy of controls.

## **Section IV Laboratory Biosafety Criteria (pages 32-59)**

Section IV introduces the laboratory biosafety level criteria. The essential elements begin with Biosafety Level 1 (BSL-1). From this base level, new criteria are added to describe and define Biosafety Level 2 (BSL-2), Biosafety Level 3 (BSL-3) and Biosafety Level 4 (BSL-4). Criteria for differing biosafety levels account for the agent being used, special practices, primary barriers, personal protective equipment and facility features. The four levels are organized in ascending order by the degree of protection provided to personnel, the equipment and the community. Each successive biosafety level contains and builds upon the recommendations of the preceding level(s).

### **Biosafety Level 1 (BSL-1) (pages 32-36)**

BSL-1 is suitable for activities utilizing well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential risk to laboratory personnel and the environment. Special practices involve standard microbiological practices, appropriate training and hazard communication, self-identification of any pre-existing conditions that may cause an individual to have an increased risk for infection, the laboratory having a safety manual, signage posted at laboratory doors when infectious materials are present, wearing and proper use of gloves, hand-washing, no eating drinking, smoking or applying cosmetics, sharps safety practices, decontamination of work surfaces and infectious materials before disposal, an integrated pest management plan and prohibiting any animals or plants not associated with the activities in laboratory spaces are additional criteria.

Notably, the BMBL-6 added the following Microbiological Standard Practices to work in BSL-1:

1. having a biosafety manual specific to the facility,
2. restraining long hair,
3. added details for glove recommendations,
4. decontamination and waste handling,
5. the use of needle-safe devices when it is necessary to remove a needle from its source.

An occupational risk assessment has also been included to BSL-1 for exposure to allergens when there is animal work involved. Facility requirements now include adequate illumination.

### **Biosafety Level 2 (BSL-2) (pages 37-43)**

BSL-2 is suitable for activities involving agents associated with human disease and pose moderate risk to personnel and the environment. Special practices include limiting access to laboratory spaces and integration of an occupational health program. In addition, all procedures that may generate an aerosol or splash are conducted in a biosafety cabinet (BSC). A validated process is recommended for decontamination of laboratory equipment. Lastly, it is recommended that consideration be given to respiratory protection during the risk assessment process.

### **Biosafety Level 3 (BSL-3) (pages 43-51)**

BSL-3 activities involve work with indigenous or exotic agents that may cause serious or lethal disease through the inhalation route of exposure. Special practices include controlled access and that the removal of viable material from the laboratory is performed using primary and secondary containers. Viable materials are only opened in a biosafety cabinet at BSL-3 or higher. Procedures with infectious materials are conducted in a biosafety cabinet. Primary barriers and personal protective equipment include the use of a biosafety cabinet for all viable agents, solid front gowns, scrubs, or coveralls, two pairs of gloves when appropriate, protective eyewear and respiratory protection as needed. BSL-3 areas have physical separation from access corridors, access through two consecutive self-closing doors, and a hands-free sink near the exit. A ducted air ventilation system with negative airflow into the laboratory space and the availability of an autoclave, preferably in the lab space, are included in secondary barriers. Changes to the BMBL-6 include additional details to reporting occupational exposures and participation in a respiratory program (if there is need for respiratory protection), transfer of samples to lower containment, and verification of decontamination procedures. In addition, BMBL-6 includes clarification related to annual testing of facilities to ensure performance of the unit.

### **Biosafety Level 4 (BSL-4) (pages 51-69)**

BSL-4 activities involve work with dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening diseases that are frequently fatal, for which there are no vaccines or treatments, as well as related agents with unknown risk of transmission. Special practices include clothing change before entry, daily inspections of essential containment and life support systems and all waste being decontaminated prior to removal from the laboratory along with shower-out procedures. Special practices include the use of full-body, air-supplied, positive-pressure suits. Entry to these spaces is sequentially through airlocks with airtight doors. Walls, ceilings, floors are

to form a sealed internal shell. Dedicated, non-recirculating ventilation systems are required, along with a double-door pass-through autoclave.

## **Section V – Vertebrate Animal Biosafety Level Criteria for Vivarium Research Facilities (pages 70-117)**

Section V follows the basic organization and scope found in BMBL-5 Section I. The Introduction to Section V includes a paragraph which describes the training required in animal welfare, biosafety practices, PPE selection and the use of containment equipment. This section also identifies the responsibilities of the Biosafety Officer (BSO) and the Institutional Biosafety Committee (IBC) for protocol review and safety policies for the protection of personnel.

### **Animal Biosafety Level 1 (ABSL-1) (pages 71 – 78)**

The introduction to the section covering ABSL-1 is essentially unchanged from the BMBL-5. The **Standard Microbiological Practices** is essentially unchanged from the BMBL-5. The material has been reorganized and edited to provide for better association between topics. Discussion of glove usage and handwashing was expanded by moving materials on these subjects from the **Safety Equipment** section in the BMBL-5. to this section. New guidance was added to this section identifying the risk associated with long hair and a requirement for proper restraint in the BSL-1 laboratory. The authors also added a note stating that additional PPE may be required for work with large animals.

### **Animal Biosafety Level 2 (ABSL-2) (pages 78 – 87)**

The **Standard Microbiological Practices** section has been reorganized in keeping with the changes identified in the ABSL-1 section above, including the requirement for long hair being properly restrained while working in the ABSL-2 facilities. The **Special Practices** section was reorganized and edited slightly. This section includes new guidance requiring verification of decontamination practices. The **Safety Equipment** section describes the use of ventilated caging and, as was described for ABSL-1, the use of gloves and handwashing was removed from this section and placed in the discussion of standard microbiological practices above. The authors also add a note stating that additional PPE may be required for work with large animals. The **Animal Facilities** section now includes validation of any alternative process for carcass decontamination and disposal.

### **Animal Biosafety Level 3 (ABSL-3) (pages 87 – 98)**

The **Standard Microbiological Practices** section has minor edits and reorganization. Notably, it includes guidance for the proper restraint of long hair while working in the ABSL-3 and details related to sharps policy and decontamination procedures for waste and equipment. **Special Practices** include added emphasis on decontamination of equipment, rooms, and waste. Details were added to the verification of decontamination processes on a routine basis. Two practices were deleted in the BMBL-6, first the collection of baseline serum samples and second, the use of eye protection if wearing contact lenses. Similar to ABSL-2, this section includes additional guidance that additional PPE may be required for persons working with large animals. Lastly, the **Animal Facilities** section now indicates that enhanced containment may be required for environmental or personnel protection based upon risk assessment as well as local, state, or federal regulations. Similar to ABSL-2, alternative processes for decontamination and disposal of carcasses must be validated.

### **Animal Biosafety Level 4 (ABSL-4) (pages 98 – 118)**

The **Standard Microbiological Practices section** indicates that waste including carcasses, animal bedding and tissues must be decontaminated by verifiable and validated procedures before removal from the ABSL-4 facility.

**Safety Equipment (Primary Barriers and Personal Protective Equipment).** The **Cabinet Facility** section includes item #7 which states that for Class III BSCs directly connected via a double door pass through to an ABSL-4 suit facility, materials may be placed into and removed from the Class III BSC via the suit

facility. Throughout this section where the BMBL-5 used the term “should” this has been replaced with “must” or “is”. The **Suit Facility** section contains clarification to address animal housing in open cages and large animals which cannot be housed in a primary containment system. These items are found on page 107 as Items 3 and 4 respectively.

In the **Animal Facilities Cabinet Facility** section, item 8.d. has added text to require all equipment and furnishing to be free of sharp edges and pinch points. Also, item 9, now includes adequate illumination for all activities as a safety requirement. Items 1-19 in the **Suite Facility** section are consistent with the content found in the BMBL-5 with minor edits; however, Items 23 and 24 contain new material dealing with animal housing in open caging. Item 23 describes the facilities design requirements for open caging in the ABSL-4 suit facility. Item 24 deals exclusively with loose-housed or open penned large animals and references Appendix D.

Table 3 Summary of Recommended Animal Biosafety Levels for Activities in which Experimentally or Naturally Infected Vertebrate Animals are used was **not** included in the BMBL-6

## **Section VI- Principles of Laboratory Biosecurity (pages 119-128)**

Section VI describes biosecurity for microbiological and biomedical laboratories. Biosecurity has multiple definitions and applications depending on the industry and country. For the purposes of this section, laboratory biosecurity refers to measures designed to prevent loss, theft, or deliberate misuse of biological material, technology, or research-related information from laboratories or laboratory-associated facilities. The BMBL-6 refers to the ISO 350001-2019 when defining laboratory biosecurity.

The BMBL-6 added details to the term agricultural biosecurity encompassing broader measures to prevent contamination of food, health, and the environment. Included in this version are statements pertaining to personnel vetting, personnel reliability, violence prevention programs, laboratory biosecurity training, dual-use research oversight process, cybersecurity standards, material and facility control, and accountability standards. The BMBL-6 stresses the importance in balancing biosafety and biosecurity considerations when developing institutional policies or finding alternative solutions that blend the two.

Lastly, the Elements of a Laboratory Biosecurity Program remain essentially the same with a few exceptions: 1) The Personnel Management element includes clarification on personnel reliability and vetting/screening policies and 2) the objective of Information Security was expanded to include data integrity.



## **Section VII—Occupational Health Support for Biomedical Research (pages 130-141)**

Section VII on Occupational Health (OH) has been revised with an explicit focus on a risk-based approach early and throughout the text. The section formally describes the need for, and the requisites of, an OH provider and OH program tailored to meet research needs. The title has been modified to include Occupational Health “Support for Biomedical Research” and removing “...and Immunoprophylaxis” broadening the description of Occupational Health appropriate to research.

The BMBL-6 Section VII introductory text is reduced to one paragraph which rapidly and clearly states the need for an appropriate Occupational Health Provider and biohazard-related Occupational Health Program that is risk-based with a focus that distinguishes it from, yet complements, the broader Occupational Health institutional program that serves all employees.

Text additions are quickly evident in the “Framework for Occupational Health Support of Biomedical Research” where five new subsections address the establishment, the conduct, and the compliance required of Occupational Health Programs. The varied approaches to meeting the Occupational Health need, the stakeholder collaboration, the frequent subject matter experts required, plus quality assurance are still included, previously in the introductory text, yet in a clearer presentation.

“Elements of an Occupational Health Program Supporting Biomedical Research” is a revision of the similarly titled portion in BMBL-5. Five new subsections are added to the previous for a total of eight topics. The risk of exposure (RoE) and risk of adverse health consequences or disease (RoD) are new concepts introduced in the subsection “Clinically-Oriented, Post-Exposure Risk Assessment”.

The elevated risks of handling RG3 and RG4 agents is woven throughout various portions of the text, such as under the OH program design, and is elaborated further in the expanded “Occupational Health support for High and Maximum Containment”, replacing the separate BSL-4 OH section in BMBL-5.

## **Section VIII-A: Bacterial Agents (pages 148-191)**

The introduction specifically notes that there are multiple resources for agent information and that the BMBL-6 is just one of those that is used by the Biosafety Professional.

***Bacillus anthracis***: The BMBL-6 updated the information related to mortality rates caused by anthrax. New information was added regarding pathogenicity of Anthrax from known exposures based on the route of exposure and post-exposure treatment. The *Special Issues* section includes advice on possible

misidentification using automated systems such as MALDI-TOF MS. The *Vaccines* section includes multiple revisions on the criteria for vaccination of workers depending on the job activity. This section also includes new available treatments. Lastly, the *Bacillus cereus* biovar *anthracis* was included as a Select Agent.

***Bordetella pertussis*:** The BMBL-6 updated the general information about *Bordetella pertussis* by including the option to be diagnosed via molecular methodologies. The natural modes of infection were updated to reflect the recent global increase of cases and circulation of *B. pertussis* in vaccinated communities. Of note, the BMBL-6 included direct contact as a hazard with additional details on survival of the bacteria on surfaces. It also updated the availability of pertussis vaccines to adults.

***Brucella species*:** The major update to this microbe was reflected in the classification of 10 species divided into terrestrial, marine, or unknown origin. *B. maris* was removed from the list but added *B. delphini*, *B. pinnipedialis*, and *B. ceti*. The occupational infections attributed to *Brucella* sp were updated to include LAIs due to mishandling and misclassification of the bacteria. The *Special Issues* section includes advice on possible misidentification using automated systems such as MALDI-TOF MS. The *Vaccines* section was updated to reflect current potential vaccines against *Brucella*.

***Burkholderia mallei*:** The former name (*Pseudomonas mallei*) was removed in the BMBL-6 and added general information related to mortality rates. The natural modes of infection were expanded to include pulmonary infection, bacteremia, etc. Laboratory safety information about survival of *B. mallei* up to 30 days in water at room temperature was added. The reference to procedures conducted outside the BSC using respiratory protection was removed in the BMBL-6. The *Special Issues* section includes advice on possible misidentification using automated systems such as MALDI-TOF MS.

***Burkholderia pseudomallei*:** The former name (*Pseudomonas pseudomallei*) was removed in the BMBL-6. Multiple updates were included to this section related to current epidemiological numbers, occupational infections. A significant update pertains to the laboratory safety and containment recommendations shifting most handling of contaminated samples to BSL-3 containment; while BSL-2 is recommended for inoculation of cultures from potentially infectious clinical samples. The *Special Issues* section includes advice on possible misidentification using automated systems such as MALDI-TOF MS.

***Campylobacter species*:** The general information was updated to indicate that *Campylobacter* species are involved not only with gastrointestinal infections, but also bacteremia, and sepsis. Molecular testing is available for detection in addition to the conventional isolation and culturing methodology. The infectious dose by ingestion was lowered to 350–800 organisms. The natural modes of infection include now additional routes such as person-to-person and groups at risk.

***Chlamydia psittaci*, *C. trachomatis*, *C. pneumoniae*:** The BMBL-6 includes multiple updates for these bacteria. The general information includes updates on the current taxonomic classification. It includes one additional occupational infection when handling fetal membranes. Multiple edits to the section on laboratory safety were made, most notably the containment needed for handling non-avian strains of *C. psittaci* may be BSL-2 following BSL3 practices. Lastly, vaccination against *Chlamydia* spp was included as not available.

***Clostridium botulinum* and neurotoxin-producing species of *Clostridia*:** This section was re-named in the BMBL-6 to include *C. botulinum*. The information about the toxin [lab safety, vaccines, post-exposure treatment] has been moved to Section VIII-G.

***Clostridioides* (formerly *Clostridium*) *difficile*:** *Clostridioides difficile* in a new addition to the BMBL-6. It is the most common cause of infectious diarrhea in hospitalized patients. This section includes a documented occupational infection. The natural modes of infection are discussed, transmission primarily occurs through the fecal-oral route or hand-to-hand contact. Laboratory safety includes containment at BSL-2 practices, containment equipment, and facilities are recommended for all manipulations. ABSL-2 facilities are recommended for in vivo studies. There is a requirement for CDC and/or USDA importation permits.

***Clostridium tetani* and Tetanus toxin:** Very minor changes or updates were made to the information for *Clostridium tetani* in the BMBL-6. The information about the number of cases of tetanus reported to CDC from 1998 through 2000 was updated to 233. A statement was added that Tetanus is considered a medical emergency and treatment with human tetanus immune globulin is recommended.

***Corynebacterium diphtheriae*:** The BMBL-6 includes updates to the agent information, notably, the gene for the exotoxin produced by the *C. diphtheriae* can also infect non-toxigenic strains. An additional occupational infection caused by *C. ulcerans*, a zoonotic pathogen, was included in the BMBL-6. Lastly, the natural modes of infection have additional information on how long *C. diphtheriae* is present in nasopharynx and skin lesions.

***Francisella tularensis*:** The BMBL-6 has updates to the agent information related to the incubation period and symptoms. This section also includes the classification of *F. tularensis subsp. novicida* as a separate species. The Laboratory Safety section refers to the Public Health Agency of Canada's Pathogen Safety Data Sheet for *F. tularensis* for survival times on different surfaces and updated the list of strains with reduced virulence that may be handled at BSL-2. This version of the BMBL references a vaccine currently under FDA review for tularemia. The Special issues includes a cautionary statement that samples processed using MALDI-TOF MS are not directly spotted on plates in the open and are extracted instead to kill viable bacteria prior to analysis.

***Helicobacter species:*** There are very minor changes to the information of *Helicobacter* species in the BMBL-6. The number of recognized species was updated to 37 (including 14 that were isolated from humans).

***Legionella pneumophila and other Legionella spp.:*** The agent information for *Legionella* spp. updated the number of species, subspecies, and serogroup. One probable case of human-to-human transmission was referenced under the Occupational Infections.

***Leptospira:*** The agent information for *Leptospira* updated the number of species including pathogenic and saprophytic species. The BMBL-6 also includes updates on the time this microbe persists on certain surfaces and one reference to a potential inhalation of contaminated droplets of urine or water.

***Listeria monocytogenes:*** The BMBL-6 has minor revisions through this section. Notably, the “should” used when discussing that pregnant women are advised of the risk of exposure to *L. monocytogenes* was reworded to “it is recommended”.

***Mycobacterium leprae:*** The BMBL-6 added *M. lepromatosis*, a related species, that can cause a similar disease to leprosy. This update also includes other modes of transmission involving animals and humans and a reference to recent increased cases of leprosy in the US. However, the inadvertent human-to-human transmission by accidental needle stick included in the BMBL-5 was removed from the new edition. Endemic animal forms of the disease have also been described from related bacterial species.

***Mycobacterium tuberculosis complex:*** The BMBL-6 added recently described species *M. canettii*, *M. mungi* and *M. orygis*. The Occupational Infections section includes now a reference to the multidrug-resistant (MDR) and extensively resistant (XDR) strains as being of particular concern. Additional modes of infection include latent infections that may reactivate and disseminated tuberculosis. The BMBL-6 includes following BSL-3 practices for animal studies (rodents) when conducted in ABSL-2 containment. Lastly, the surveillance section includes the use of the FDA-approved Interferon Gamma Release Assay (IGRA) as an alternative to PPDskin testing when skin tests have been negative.

***Mycobacterium spp. other than M. tuberculosis complex and M. leprae:*** The information for these organisms has been updated in the BMBL-6 to reflect current knowledge of this species. > 150 *Mycobacterium* species have been identified. Some of these species are slow growing while others grow rapidly. Mycobacterial isolates not part of the *M. tuberculosis* complex are now called nontuberculous *Mycobacteria* (NTM) or *Mycobacteria* other than tuberculosis (MOTT). New to the BMBL-6 is the reference to an LAI with *Mycobacterium* spp. via needle stick while conducting an experiment in mice.

***Neisseria gonorrhoeae***: Minor updates were made to this section to update incidence rates of gonorrhea. A special issue was included to reference the emergence of an extensively drug-resistant strain (XDR) and other antimicrobial resistant strains. As such, certain antibiotics are not recommended for treatment of uncomplicated gonorrhea.

***Neisseria meningitidis***: The BMBL-6 updated the agent information for *N. meningitidis* to include one additional serogroup X and specific potentially infected source materials such as fluids from sterile sites. The laboratory safety practices emphasize handling bacterial cultures and inoculation of clinical specimens inside the BSC. This update also includes the *N. meningitidis* vaccines currently available and recommendations for laboratorians potentially exposed to this bacteria.

***Salmonella* serotypes, other than *S. enterica* serotype Typhi (*S. Typhi*)**: The BMBL-6 includes recommendations for antimicrobial therapy, additional information about modes of infection and the problem of antimicrobial resistance of *Salmonella spp.* This update also mentions that vaccines against non-typhoidal strains are not available.

***Salmonella enterica* serotype Typhi (*S. Typhi*)**: This update includes the infectious dose for *S. Typhi* at <1000 organisms, instead of <10<sup>3</sup> organisms. The laboratory safety and containment recommendations removed the specifics associated with PPE use such as the splash shields, face protection, gowns, and gloves.

**Shiga toxin (Verocytotoxin)-producing *Escherichia coli***: This agent summary is limited to Shiga toxin-producing *E. coli*. The BMBL-6 updated the number of *E. coli* species, the survival of the bacteria on environmental surfaces. Importantly, the details included in BMBL-5 for laboratory safety have been condensed to use of PPE, handwashing, and decontamination of surfaces.

***Shigella***: Minor updates were incorporated in the introductory paragraphs to isolation methods from infected source materials.

***Staphylococcus aureus* (Methicillin-Resistant, Vancomycin-Resistant, or Vancomycin-Intermediate)**: This is a new agent summary added in the BMBL-6. *S. aureus* is a Gram-positive bacterium and is found to be associated with minor to severe disease. Ingestion of food containing enterotoxins results in infection. No vaccines are available for human use. Lab workers can become exposed to the agent through broken skin, mucous membranes, contaminated surfaces, parenteral inoculation, ingestion and while handling cultures. BSL-2 practices, containment equipment, and facilities are recommended for all activities utilizing known/potentially infected clinical materials and cultures. ABSL-2 facilities are recommended for studies utilizing infected laboratory animals. Importation of this agent requires CDC and/or USDA import permits.

***Treponema pallidum***: The description of *Treponema pallidum*, the causative organism for syphilis, bejel, and yaws has remained unaltered in BMBL-6 with the exception that the nomenclature was updated for the subspecies (example *T. pallidum* subsp. *pallidum*).

***Vibrio species***: The BMBL-6 has updated the agent summary for the *Vibrio* genus, which harbors several species including *V. cholerae*, a common cause of human enteritis. Fatal cases of septicemia have been included as natural modes of infection in immunocompromised or individuals with pre-existing conditions. Importantly, references to LAIs of *V. cholerae* or *V. parahaemolyticus* associated with the use of syringes, spill clean-up, or handling of infected animals are included in this edition. Lastly, the **Vaccines** section has been updated to reflect current availability and recommendations.

***Yersinia pestis***: New information was added about the mortality rate and the different manifestations of the disease. A LAI involving an attenuated strain KIM D27 was fatal. Attenuated strains such as A1122 can be handled using BSL-2 practices, containment equipment and facilities. The BMBL-6 includes caution when automated systems are used to identify this bacteria to avoid misidentification.

## **Section VIII-B: Fungal Agents (pages 212-219)**

Section VII-B comprises summaries for fungal agents that include *Blastomyces dermatitidis* and *Blastomyces gilchristii*, *Coccidioides immitis* and *Coccidioides posadasii*, *Histoplasma capsulatum* and *Sporothrix schenckii* species complex. Each agent summary details occupational infections, natural modes of infection, laboratory safety and containment recommendations and any special issues. These agents require an import permit and a domestic transfer permit.

***Blastomyces dermatitidis*** is a dimorphic fungus that exists in nature and laboratory cultures at room temperature as a filamentous mold with spores that are infectious particles. New to the BMBL-6 is the addition of *Blastomyces gilchristii* which has recently been recognized as a novel species. Spores, or conidia, convert to large budding yeasts under appropriate culture conditions in the laboratory and in the parasitic phase in warm-blooded animals. Infections occur when spores are inhaled, or the yeast form is injected. LAIs have occurred through parenteral inoculation with tissues or cultures containing yeast forms, as well as through inhalation of spores. At least 11 reported LAIs with two fatalities have occurred. Natural modes of infection require common exposure from a point source from the environment and human-to-human transmission occurs rarely via perinatal or sexual transmission. BSL-3 practices are recommended for samples that contain spores. BSL-2 and ABSL-2 practices are recommended for diagnostic and yeast-form cultures.

***Coccidioides*** species are dimorphic fungal pathogens that exist in nature and lab cultures at room temperature as filamentous molds, with asexual spores that are the infectious particles. Occupational

exposure has been associated with high dust exposure in endemic regions. LAIs tend to result in clinical disease whereas infections in nature tend to be asymptomatic. Single spores can produce infections by the respiratory route, though most infections from the environment are subclinical. BSL-3 practices are recommended for propagating and manipulating spores, processing soil known or suspected to contain spores or animal studies where challenge is via inhalation or the pulmonary route. BSL-2 practices are recommended for clinical specimens and ABSL-2 practices are recommended for animal studies involving the parenteral challenge.

***Histoplasma capsulatum*** is a dimorphic fungal pathogen that exists in the lab culture setting as filamentous mold with asexual spores. Specific hazards include increased risk to immunocompromised individuals. LAIs tend to be common among diagnostic work, from handling mold for cultures and spray to the eyes. Spores are resistant to drying and may remain viable for long periods of time. Natural modes of infection require a point source and infections are not transmissible from person-to-person. BSL-3 practices are recommended for propagating sporulating *H.capsulatum* in the mold form or environmental samples suspected to contain spores. BSL-2 and ABSL-2 practices are recommended for clinical specimens and a biosafety cabinet is used for any samples where dimorphic fungi are identified.

***Sporothrix schenckii*** species are dimorphic fungal pathogens that exist in nature and in lab cultures as filamentous mold with asexual spores. Large outbreaks of sporotrichosis have been documented in environments where exposed to soil or plant material containing the fungus. Occupational exposure cases have been associated with local skin or eye infections in lab personnel. BSL-2 and ABSL-2 practices are recommended for handling of clinical specimens and a BSC is used for any culture identified as dimorphic fungi.

The BMBL-6 removed *Cryptococcus neoformans* and Dermatophytes agent summaries. Instead a new table, Table 1 Miscellaneous Yeast and Mold, has been added at the end of the section describing occupational and natural mode of infection and the biosafety level for *Candida* species, *Cryptococcus neoformans* and *C. gattii*, Dermatophyte molds, Hyaline molds, *Talaromyces marneffeii*, Dematiaceous molds and Mucormycete molds.

## **Section VIII-C: Parasitic Agents (pages 223-237)**

The BMBL-6 includes the same five subsections of parasites included in BMBL-5. The first two subsections include taxonomically diverse protozoal groups categorized according to lifestyle/ mode of infection. These two subsections include Microsporidia, which are noted actually to be fungi but are grouped with protozoal parasites for historical reasons. The last three categories represent distinct taxonomic groups from the phyla Platyhelminthes (trematodes and cestodes) and Nematoda respectively. A history of LAIs is noted for most subgroups. Known and possible routes of LAIs (e.g.

ingestion, percutaneous exposure, arthropod vector bite, etc) are noted for each category. For all parasite groups except nematodes, BSL-2 and ABSL-2 are specifically recommended when handling infective stages; a specific biocontainment level is not recommended for nematode parasites (but BSL-2 / ABSL-2 may be inferred). The need for arthropod containment per Appendix E when infected vector arthropods are present is noted. Specific inactivation procedures using bleach, peroxide, etc are recommended for certain organisms. Special CDC, USDA and/ or DoC permits may be required for importation and shipping for most parasites.

The BMBL-6 includes additional species of Free-Living Amoeba (FLA) and describes the medical conditions associated with FLA, and potential sources of infection in the workplace.

### **Section VIII-D: Rickettsial Agents (pages 239-244)**

This section is in the same order as in the BMBL-5, immediately following Parasitic Agents. All headings found within are also in the same order as the previous edition.

The Rickettsial Agents section begins with *Coxiella burnetii*, an intracellular pathogen and Select Agent. There is a brief description of *C. burnetii* followed by the same headers used throughout Section VIII. There are two noticeable modifications to the BMBL-6 from the BMBL-5. First, the header Laboratory Safety and Containment Recommendations now includes a paragraph regarding cell-free media that has been recently developed and more widely disseminated. This header also contains a brief statement regarding a plaque purified clonal strain that is Select Agent exempt and can be used safely following BSL-2 conditions. The second modification to the *Coxiella burnetii* section is that the mention of a Q-fever vaccine in the IND phase has been removed.

Following *C. burnetii*, the authors coalesce all other *Rickettsia* spp. into a single remaining section. There were some minor modifications to this section to reflect newly identified Rickettsial species and safety practices. Specifically, *R. philipii* and *R. parkeri* have been added. In terms of the Safety heading, a large paragraph describing BSL-2 with enhanced practices when performing laboratory-based research using Rickettsial species has been added.

### **Section VIII-E: Viral Agents (pages 247-280)**

**Hantavirus:** The Laboratory and Safety Recommendations include the use of these viruses in rodent species inducing chronic infections should be conducted at ABSL-4. Similarly, cell-culture propagation should be conducted at BSL-3 following BSL-3 practices, and handling of serum or tissue samples should be handled at ***BSL-2 following BSL-3 practices, containment equipment, and procedures***. Lastly,



potentially infected samples should be handled at ***BSL-2 following BSL-3 procedures and practices***. The special Issues subsection added the potential permit required by the Department of Commerce for exporting this virus.

**Hendra Virus (formerly known as Equine Morbillivirus) and Nipah Virus:** The BMBL-6 contains updated information about transmission of this virus and the new henipa virus, Cedar virus. Human-to-human transmission is included as a natural route of transmission. Laboratory and Safety Recommendations are provided for Cedar virus. Special Issues include mention of the Hendra vaccine available for horses in Australia

**Hepatitis A Virus, Hepatitis E Virus:** This section includes updates for Hepatitis E virus (HEV) as its genus Orthohepevirus has been assigned to the Hepeviridae family and recognizes four genotypes infecting humans. Occupational infections now have expanded HEV genotype 1 as a risk for pregnant women. The update also acknowledges the potential risk of exposure to HEV-infected pigs, rabbits, or macaques. The Natural Modes of Infection was updated to include the modes of transmission for each HEV genotype. The Vaccine section was updated to include a HEV vaccine currently available in China.

**Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus:** The BMBL-6 removed the alternate name “non A non B” virus for Hepatitis C (HCV). The special Issues subsection added the potential permit requirement by the Department of Commerce for exporting this virus to another country.

***Macacine alphaherpesvirus 1 (Herpesvirus Simiae, Cercopithecine herpesvirus 1, Herpes B Virus)***: The name of the Herpes B virus was updated in BMBL-6 to reflect its current scientific name. Herpes B virus was removed from the Select Agent list, thus the notation of it being a Select Agent was removed in the BMBL-6, however, the permit requirement by the Department of Commerce was added.

**Human Herpes Virus:** This section has been updated in the BMBL-6 to reflect current knowledge related to clinical presentation, modes of natural transmission and treatment. Importantly, the following sentence was added for handling Epstein Barr Virus: “*Autologous transformation of B cells using EBV should not be performed*”.

**Influenza Viruses:** The Influenza summary has been significantly modified to reflect newly identified serotypes and current research. Laboratory Safety and Containment recommendations are provided for **Seasonal Human Influenza Viruses and Zoonotic and Animal Influenza A Viruses** in addition to the existing Non-Contemporary Human Influenza Viruses, 1918 Influenza A (Pandemic Virus), Highly Pathogenic Avian Influenza A Viruses (HPAI) and other Influenza Recombinant or Reassortant Viruses. The need for risk assessment is emphasized when handling wild-type human influenza A (H1N1) and A

(H3N2) as they may not circulate all the time, a case in point is discussed with the pre-2009A(H1N1) and the A(H1N1)pdm09, as pre-pandemic and pandemic viruses, respectively.

**Lymphocytic Choriomeningitis Virus:** Added to this section in the BMBL-6 is the inclusion that women in childbearing age should be made aware of risks posed by LCMV or rodents potentially infected by LCMV.

**Poliovirus:** The Poliovirus section now includes WHO GAPIII requirements for Type 2 and WPV3 and Type 1 and OPV3.

**Poxviruses:** BMBL-5 stated that the NIH Guidelines assessed the risk for handling certain attenuated strains at BSL-1, if no other human orthopoxviruses were being used. The BMBL-6 indicates that lower containment for attenuated poxviruses and vectors *may* be considered. It also added that for research subject to the NIH Guidelines, approval to lower containment from BSL-2 must be requested from the NIH Office of Science Policy.

**Rabies Virus and related Lyssaviruses:** A new table has been included in this section including Lyssavirus species and the recommended biosafety level (page 272).

**Retroviruses, including Human and Simian Immunodeficiency Viruses (HIV and SIV):** The BMBL-6 contains updated pathogen information for HIV, SIV, Simian foamy viruses (SFV), Simian Retrovirus (SRV), and the SIV/HIV genetic recombinant virus (SHIV) (page 276).

**Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS)**

**Coronaviruses:** MERS has been included in this section. The pathogen information and occupational infections for SARS has been updated.

**Section VIII-F: Viral Agents Arboviruses and Related Zoonotic Viruses (pages 292-330)**

Several modifications to this section were made. Table 3 lists Arboviruses and Hemorrhagic Fever Viruses by family and genus, recommended biosafety level and the antigenic group (page 308). Note that the HEPA filtration on lab exhaust information has been removed from this table. Table 4 is an arthropod-only table including family, genus, recommended biosafety level and isolate (page 327). Table 5 lists which viruses are handled at BSL-3 with HEPA filtration on lab exhaust (page 329).

Importantly, **West Nile** and **St. Louis Encephalitis** viruses are now recommended to be handled in BSL-2 facilities, containment equipment, and practices. However, a risk assessment is needed if working with extremely high titer of virus or aerosol-generating procedures. The Central European Tick-Borne Encephalitis Virus (TBEV-CE) has been reclassified to require BSL3 containment, provided that at-risk personnel are immunized.

## **Section VIII-G: Toxin Agents (pages 334-347)**

The information provided on Toxin Agents has been updated and revised. Overall, more detailed and updated information is now provided on the various toxic agents included.

The information on Botulinum neurotoxin (BoNT) includes more background information and more details on symptoms and diagnostics. More specific information is included on laboratory containment and PPE recommendations. More information on the stability of the bacteria and toxin is included. A statement on recombinant materials has been added. Updated vaccination and post-exposure treatment information included.

The information on Staphylococcal enterotoxins (SEs) has been updated to include more background information on the toxins, and more information on the different routes of exposure. More specific symptoms of intoxication are listed. The PPE recommendations have been updated to include safety goggles (based on risk), and ocular exposure is included as a risk.

The information on Ricin toxin has been updated and expanded to include more information on latency periods and an expanded list of symptoms of intoxication. An updated list of diagnostic tests is included. The information on engineering controls has been simplified, and methods for toxin inactivation have been included. There is mention of a vaccine candidate.

The information on selected Low Molecular Weight toxins (LMWs) has been updated, including updates on species information for certain toxins. Information on domoic acid (a neurotoxin) has been added. Updated information on the presence and stability of marine toxins in food, and the metabolism and excretion of said toxins included.

For all the toxins, there is updated information on the regulation of certain toxins, including references to the Federal Select Agent Program (FSAP).

## **Section VIII-H: Prion Agents (pages 355-363)**

The BMBL-6 includes updates on the agent summaries, and containment (for example handling of prions from human source at BSL-2 or higher). The special section includes details for handling of unfixed prion-containing samples collected during autopsy in a BSL-2 facility with ***restricted access, additional PPE, and dedicated equipment.***

The inactivation section includes details of acceptable methods such as enzymatic treatment and hydrogen peroxide.

Lastly, the BMBL-6 includes the following statement before the description of decontamination procedures: ***The FDA has not yet approved any product for decontaminating, disinfecting, or sterilizing prions. The methods described are considered research use only.***

## **Appendix A – Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets. (pages 367-397)**

Appendix A has been revised to include changes reflected in the NSF/ANSI 49-2018 as well as to provide additional guidance for improved selection, installation and use of biological safety cabinets (BSCs). Changes have been made to all Parts (previously referred to as Sections) and key updates are briefly summarized as follows:

**Part 1. Introduction:** The BMBL-6 includes prions among the hazardous material which may be safely manipulated in a biosafety cabinet.

**Part 2. High Efficiency Particulate Air (HEPA) Filters and the Development of Biological Containment Devices:** ULPA filters are introduced and their efficiency level is compared with HEPA filters. Different media types such as polytetrafluoroethylene (PTFE) used for the construction of HEPA and ULPA filters are included.

**Part 3. Biological Safety Cabinets:** The use of a canopy connection and an exhaust alarm is included for Class I BSCs when hazardous gases and vapors are used. Class II/Type C1 cabinets are introduced as well as the use of canopy connection (removed hard ducted) as the proper method of connecting Types A1, A2 or C1 to the building exhaust system. Class II/Type B1 cabinets are to be directly connected, preferably to a dedicated independent exhaust system. A pressure independent monitor alarm (not usually installed by the manufacturer) to signal supply fan failure should also be installed and it is connected to emergency power. The mechanical design and air balance testing of laboratory exhaust

systems for Class IIB BSCs must use the Concurrent Balance Values (CBV) as indicated in the NSF/ANSI 49. A description of the CBV testing is included. A risk assessment is recommended prior to using a Class II/B2 cabinet because of the associated costs of operation. The use of a dedicated exhaust system for Class III cabinets is recommended.

The preparation of nucleic acids for PCR was added to the potential uses of a Vertical Flow Clean Bench.

#### **Part 4. Other Laboratory Hazards and Risk Assessments**

References are made to OSHA's Permissible Exposure Limits for assessing the risks associated with hazardous chemicals and to the Public Health Agency of Canada for data on microorganisms associated with LAIs. Chemical fume hoods are recommended for work involving volatile chemicals and where biohazard containment is not required. The BMBL-6 added Class II/C1 BSCs for working with many liquid chemicals, non-volatile antineoplastic agents, etc., however these must be properly canopy vented (hard ducting of these has been removed). The bag-in/bag-out method for removal of chemically contaminated filters is included in order to minimize the risk of exposure.

#### **Part 5. BSC use by the Investigator: Work Practices and Procedures**

The application of ergonomics principles are introduced in the BMBL-6 for proper back and feet support as well as the use of elevated ergonomic arm/elbow rests on the grilles of BSC to minimize airflow disruption. BSCs equipped with specialized equipment, which have been installed by the manufacturer, must be field tested in accordance with the manufacturer's testing methodology to ensure containment in accordance with NSF/ANSI 49-2018. When new or different equipment is placed inside the cabinet, smoke testing is required to ensure containment as per NSF/ANSI 49-2018. The manufacturer should provide the user with the certification testing methodology.

A risk assessment is recommended prior to allowing two people to work in a BSC at the same time. Where night setback modes are used, BSCs are interlocked with laboratory supply and exhaust systems to maintain negative air balance. The use of touch plate micro burners with pilot lights are to be used in BSC only with prior management approval and after a thorough risk assessment has been completed.

Disinfectants used for surface decontamination of BSCs must be suitable to laboratory needs. Clarification is provided on the use of disinfectants in liquid waste containers, to ensure that the disinfectant is at a final concentration which is effective against the microorganisms in use, when the container is filled to the designated capacity. Procedures for spill clean-up (inside the BSC) have been revised.

## Part 6. Facility and Engineering Requirements

When building exhaust is used to vent Class II/B cabinets in a BSL 4 facility, the system must be designed using the CBV and must have sufficient capacity to maintain exhaust flow. The use of ultraviolet (UV) light should not be the only method of disinfection for BSCs. This section includes recommendations on what to do when UV light is used. An additional requirement for decontamination of bag-in/bag-out filters is included.

## Part 7. Certification of BSCs

- The Federal Standard 209 has been replaced with ISO 1464-2015.
- Reference to NSF/ANSI 49 has been removed and replaced with NSF/ANSI 49-2018.
- Details of field testing have been removed and referenced in NSF/ANSI 49-2018 Annex F.
- Tables 1 and 2 (Selection of BSCs; Comparison of BSCs) have been revised.
- Tables 3 and 4 (Field performance testing; Applicable containment tests) ) have been removed.

## Appendix B – Decontamination and Disinfection of Laboratory Surfaces and Items (pages 400-410)

Appendix B highlights the need for the appropriate selection of disinfectants and potential risk of exposure to undiluted solutions. This Appendix now includes the ***Antimicrobial Products—U.S. Regulations*** section to describe the multiple sources of oversight.

The BMBL-6 includes additional sources of environmental contamination, for example, generation of aerosols through specific procedures or splashes. In addition, hand hygiene and proper use of PPE are included as factors in preventing transmission.

The term “Cleaning” is added to the BMBL-6 under this section as a pre-requisite to disinfection or sterilization.

Contact (exposure) time has been added as the fifth factor affecting **Disinfection**.

The BMBL-6 has deleted the text that provided the background of Spaulding Classification. Similarly, the explanations of 'critical', 'semi-critical', and 'non-critical' classification of chemical germicides used on medical devices have been removed. The detailed descriptions of 'High-level Disinfection', 'Intermediate-level Disinfection' and 'Low-level Disinfection' have been removed here as well. These terms are briefly mentioned at the beginning of the Appendix B in reference to the applicable FDA regulation, Definitions of these terms/designations are in the Glossary.

The **Decontamination** section has consolidated decontamination in the Microbiology laboratory and Decontamination and Cleaning sections. **Figure 1. Descending Order of Relative Resistance to Disinfectant Chemicals** includes Prions as the highest order of resistance, it also includes enveloped and non-enveloped viruses instead of lipid and non-lipid viruses.

**Table 1** was renamed Activity Levels of Selected Liquid Chemical Disinfectants and the concentrations and activity levels have been updated.

The BMBL-6 includes specific sections on inactivation of Select Agents with reference to the Select Agent guidance on inactivation and biosafety.

Lastly, Appendix B has a section dedicated to Hand Hygiene as part of risk mitigation, it also includes the use of alcohol-based hand sanitizers.

## **Appendix C – Transportation of Infectious Substances (pages 415-421)**

Changes of note include updates to reflect new ICAO & IATA requirements , updates to outdated contact information and weblinks, and modifications to verbiage for flow, not content. Structurally, this section is very similar to the BMBL-5 with the one major change, shipment of Select Agents has become its own, stand-alone subtitle.

## **Appendix D – Biosafety and Biocontainment for Pathogens Affecting Agricultural Animals and Animals that are Loose-Housed or in Open Penning (pages 423-456)**

Appendix D of the BMBL-6 is substantially different from that in the BMBL-5, starting with a more informative title. As the expanded introduction states, this appendix “...focuses primarily on *in vitro* and *in vivo* research and diagnostic activities involving pathogens that primarily affect agricultural animals and other animal species that cannot be housed in primary containment isolators or an equivalent means of primary containment following challenge.”

The BMBL-5 provided a description of facility and work practice enhancements added to ABSL-3 to become BSL-3Ag, as well as potential facility enhancements to BSL-3 and ABSL-3 for agriculture agent permitting. The BMBL-6 includes guidance for performing risk assessments to ensure appropriate biosafety and biocontainment practices are in place. In addition, there has been both a change in terminology, Animal Biosafety Level-3 Agriculture (ABSL-3Ag) replacing BSL-3-Ag, and an addition of new terminology: ABSL-2Ag and ABSL-4Ag. Potential enhancements to non-agriculture biosafety levels for conducting research with animal pathogens for work conducted in primary containment now includes BSL/ABSL-2 and BSL/ABSL-4, in addition to BSL/ABSL-3 found in the previous edition. Similarly, potential enhancements for work with animal pathogens outside primary containment now includes ABSL-2Ag and ABSL-4Ag in addition to ABSL-3Ag. The most important, and welcome, changes found in the new edition are the sections describing potential enhancements. Not only have these been rewritten (or written for the new sections) for clarity in guidance and better applicability, but they are also presented in a logical order from lower to higher biosafety levels. The final change in this appendix is the replacement of agent summaries with tables of an expanded list of agents and recommended containment levels. These tables also include natural host range, natural routes of transmission, and environmental stability information for each agent.

## **Appendix E – Arthropod Containment Guidelines (pages 458-459)**

This section includes changes with an increased focus on risk assessment and containment levels. In addition, Appendix E in the BMBL-6 includes a citation of the most recent publication and revisions to the [Arthropod Containment Guidelines](#), Version 3.2 published in 2019 by the American Committee of Medical Entomology, a subcommittee of the American Society of Tropical Medicine and Hygiene, included as the 2<sup>nd</sup> reference for this Appendix.

## **Appendix F – Select Agents and Toxins (page 460)**

The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 and Agricultural Bioterrorism Protection Act of 2002 requires the Department of Health and Human Services and U.S. Department of Agriculture to regulate the possession, use, and transfer of select biological agents and toxins that have the potential to pose a severe threat to public health and safety and animal and plant health or products. The Federal Select Agent Program (FSAP) oversees these activities and is jointly run by the Centers for Disease Control and Prevention's Division of Select Agents (42 CFR Part 73) and Toxins and the Animal and Plant Health Inspection Service's Agriculture Select Agent Services (7 CFR 331 and 9 CFR Part 121).

The key safety and security elements of the regulations include: required registration with FSAP; security risk assessment of registered personnel, enforcement actions for violations; designation of Responsible Officials at entities; development of various plans (security, incident response, and biosafety);



requirement for approval of certain experiments; training requirements, duty to report various situations; approval of all transfer; maintenance of records and documentation; authority to inspect entities; specific exemptions and exclusions; and requirement to validate inactivation procedures.

## **Appendix G – Integrated Pest Management (pages 463-465)**

This appendix describes the importance and design of an integrated pest management program to laboratory management. This section is almost identical to its counterpart in the BMBL-5 except for minor verbiage modifications. The only addition not found in the BMBL-5 is under the FACILITY DESIGN header, where the use of the NIH Design Requirements Manual was specifically mentioned as a resource.

## **Appendix H – Working with Human, NHP, and Other Mammalian Cells and Tissues (pages 466-468)**

The BMBL-6 Appendix H provides a more detailed guidance for working with clinical or research samples which may contain bloodborne pathogens including risk assessment and risk mitigation precautions presented in six new sections: The **Bloodborne pathogens and risk assessment related to material source and type** provides a basic description of bloodborne pathogens with several common examples as a basis for the risk assessment to be performed when working with human or NHP blood or other potentially infectious materials, as well as a reference to the USA OSHA Bloodborne pathogen standard. Factors to consider during the risk assessment include **Tissue source** (human pathogens and zoonotic agents); **Cell or tissue type** (cells from different tissues and sources present different risks for oncogenesis); **Culture Type** (potential of cells maintained in culture for harboring undetected pathogens or viral genomes which may present a risk to personnel using primary cultures or cell lines permissive to various viruses known to be pathogenic to humans).

**Additional considerations** for handling human and NHP source materials include endogenous pathogens present in the specimens or which may have been introduced for experimental purposes when developing the risk assessment. Lastly, the **Risk Mitigation** section describes the use of universal precautions (BSL-2 practices, engineering controls, use of a BSC, and PPE) for handling RG-2 pathogens or samples with no known infectious agents. RG-3 and RG-4 pathogens need to be handled using higher level of containment practices and engineering controls.

## **Appendix I – Guidelines for Work with Toxins of Biological Origin (pages 470-481)**

The table of contents (TOC) for Appendix I has listed nine sections (to include references) under the “**Guidelines for Work with...**” heading with corresponding page numbers to make it simple to go directly

to a specific section by listed page number whereas the BMBL-5 only listed “Guidelines for Work with Toxins...” in the TOC. No longer used is the description of “toxins comprise a broad range of poisons”. The BMBL-6 describes properties of toxins using molecular level characteristics in the opening description.

## **Appendix J – NIH Oversight of Research Involving Recombinant Biosafety Issues (page 484)**

The spirit of this appendix is essentially the same. All changes reflect updated verbiage such as the NIH structural change from the Office of Biotechnology Activities (OBA) to the Office of Science Policy (OSP) or to match the current requirements in the NIH Guidelines which have been updated multiple times since the last BMBL. The removal of the RAC has also been addressed.

## **Appendix K – Inactivation and Verification (pages 486-501)**

The purpose of Appendix K is to describe inactivation methods or strategies that allow for the preservation of characteristic(s) relevant to further analysis or study of pathogens, viral nucleic acid sequences, or toxins and verification of inactivation.

The guidance outlines selection of an appropriate inactivation method considering the pathogen, viral nucleic acid sequences, or toxins, the targets and/or actions of the inactivation method, as well as consideration of numerous factors that have a bearing on the development of the inactivation protocol or method of removing the pathogen from the material.

Tables summarize the advantages and disadvantages of physical, chemical, chemical activated by physical treatment, natural and emerging antimicrobial strategies, and combination methods for inactivation methods.

To ensure the inactivation procedure is adequate for the conditions, process controls for validation and verification or viability testing parameters are described.

Institutional oversight, review, and approval is recommended to ensure adequate confirmation of inactivation and separation/removal procedures are developed. Documentation and recordkeeping of inactivation/verification procedures are to be developed by the entity to track materials that are moved from a higher to lower containment. Administrative controls should address appropriate risk assessment and mitigation if a failure or variance of inactivation procedure occurs and have a plan for communication, root cause analysis, and incident response.

Regular review of inactivation and verification procedures to ensure continued efficacy as well as when a failure in the procedure occurs.

For consistent inactivation, consider the reagent sources and equipment maintenance used in inactivation procedures.

Personnel executing the inactivation procedures should also be trained and demonstrate proficiency.

## **Appendix L – Sustainability (pages 504-512)**

The BMBL-6 includes suggestions for existing laboratories and new facilities to conserve resources.

## **Appendix M – Large-Scale Biosafety (pages 515-526)**

This entirely new appendix, specific for activities involving greater than 10 liters of biohazardous agents, is analogous to, but substantially different from, Appendix K the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)*. The focus of this appendix is the risk assessment process rather than a prescriptive set of requirements for four physical containment levels. The bulk of the appendix addresses risk mitigation strategies using engineering controls, work practice and administrative controls, and PPE. This section also addresses the change in manufacturing processes from fixed equipment, such as large steel fermentation tanks, to single-use equipment including the “ballroom” concept of manufacturing. Several pages are devoted to the containment requirements and example risk points associated with single-use equipment.

## **Appendix N – Clinical laboratories (pages 529-541)**

This is a new appendix in BMBL-6. Appendix N highlights the fact that a CLIA lab director is already subject to strict expectations and statutory regulations that place responsibility for positive and negative outcomes squarely on the lab director. Appendix N suggests that a biorisk management system in a clinical lab can use a system of SOPs, training, and QC that will be familiar to staff and managers for CLIA compliance. This new section in the BMBL-6 provides some practical approaches for effective BRM programs in a clinical laboratory setting while taking into consideration the unique nuances/challenges facing them such as unknown samples, point-of-care (POC) and bedside testing and unavailability of facility/resources for risk mitigation.

Notable topics in this appendix include:

- Biorisk Management
- Mitigation measures emphasizing Engineering controls, Administrative (and work practice) controls, and PPE.
- Prior experience with the 2014-2015 Ebola outbreak.
- Proactive specification of “trigger points”, such as positive cultures appearing in certain specimen types, that prompt workers to increase biocontainment.
- Leveraging an existing culture of quality control to promote a culture of safety based on SOPs, training, and emergency response plans and drills. Consideration of risk ethics, risk tolerance, and risk aversion to hazardous work at the institutional and personal level.