Interim Clinical Laboratory Guideline for Biological Safety

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DISCLAIMER: The information presented in this document is not all-inclusive and is instead a summary of the authors' interpretation of the current (as of January, 2019) requirements and regulations concerning biological safety.

1. INTRODUCTION

Clinical laboratory biosafety is an integral process that is meant to ensure safety of laboratory staff. By extension, biosafety is also meant to ensure the safety of the rest of the medical facility (including other hospital staff and patients), the community, and laboratory staff families and friends. A laboratory accident or laboratory-acquired infection could affect not only the laboratory staff but others around them.

Clinical specimens submitted to diagnostic and public health microbiology laboratories can contain microorganisms that pose safety risks to those handling the specimens themselves and any microbial cultures derived from them. These microorganisms can include nonpathogenic or moderately hazardous agents such as routinely isolated bacteria and fungi as well as higher-risk pathogens, including *Mycobacterium tuberculosis* and agents of viral hemorrhagic fever. In order to categorize the threats posed by these microorganisms to laboratory staff, various classification schemata have been developed. By and large, these systems are based on the risk of agent transmission within the laboratory, the severity of diseases caused by the agents, and the availability of specific prophylactics and anti-infective therapies. The American Biological Safety Association (ABSA) classifies microorganisms into 1 of 4 "risk groups" (RG) based upon the aforementioned criteria; these are also described in the World Health Organization (WHO) Biosafety Manual (http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/). Briefly, RG-1 encompasses biological agents not associated with disease in healthy humans; RG-2 encompasses agents that cause disease in humans, but pose only minimal or moderate risks of transmission or disease in laboratory workers; RG-3 organisms are those that are easily transmitted within the laboratory and are capable of causing serious disease in humans, but for which effective therapies are available, following exposure or for treatment of infections. Finally, RG-4 agents cause severe disease in humans and are easily transmissible, but unlike some RG-3 organisms, effective prophylactics and therapies are not available. A searchable database containing the risk group classification of microorganisms is available at the following web address: https://my.absa.org/tiki-index.php?page=Riskgroups. While knowledge of the risk group classification of microorganisms can be important, clinical laboratories should always perform risk assessments for all procedures.

In 2002, a federal law was enacted requiring the US Department of Health and Human Services (HHS) to establish a list of specific microorganisms and toxins that pose an elevated risk to human health and public safety. These agents were designated as "select biological agents and toxins," commonly referred to as "select agents," which consist of a large number of bacteria, viruses, fungi, and toxins. This list is dynamic and undergoes periodic updating as new information is learned about currently-classified agents and as novel agents emerge. An updated list of these agents is available at the following web address: https://www.selectagents.gov/SelectAgentsandToxinsList.html. Among the HHS select agents, a subset of microorganisms and toxins has been designated as "Tier 1" based on a high likelihood for use as an agent of bioterrorism. Agents used as biological weapons and high-consequence, naturally-occurring biological agents will, from here forward, be referred to as biothreat, or BT, agents. These agents are typically easy to disseminate, cause infection via respiratory exposure, and have a low infective dose. They also carry high rates of morbidity and mortality and specific antibiotic or antiviral therapies may not be available (Table 1). It should be noted that the identification of Tier 1 select agents and toxins require immediate (i.e., within 24 hours)

reporting to the Federal Select Agent Program by telephone, fax, or e-mail. A complete list of select agents, including those designated as Tier 1 BT agents by the Centers for Disease Control and Prevention (CDC) is available: http://www.selectagents.gov/SelectAgentsandToxinsList.html. Importantly, select agent tier and ABSA risk group designations are not synonymous with the biosafety level (BSL) 1-4 laboratory classification scheme.

The role of the sentinel laboratory, which includes Clinical Laboratory Improvement Amendments (CLIA)-certified clinical microbiology laboratories, is to recognize clinical specimens or isolates containing potential BT agents and other highly infectious agents of interest to public health. If the laboratory cannot rule out these agents, the specimen or isolate is referred to the appropriate Laboratory Response Network (LRN) reference laboratory for definitive identification. To effectively fulfill this role, the sentinel laboratory must be familiar with the current list of federally recognized BT agents and have protocols in place to safely handle these specimens and cultures. This includes policies for safe work practices, use of personal protective equipment (PPE), physical manipulation of specimens and isolates, conduct rule out testing, risk assessment, and training in the safe packaging and shipping of these agents. This guideline provides specific insight into these topics based on current literature and related safety recommendations with the exception of safe packaging and shipping, which is covered in the ASM document "Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: Packaging and Shipping of Infectious Substances."

Table 1. Tier 1 select agents affecting humans.[a]

Bacterial	Viral	Toxins
Bacillus anthracis *Bacillus cereus* biovar *anthracis* *Burkholderia mallei* *Burkholderia pseudomallei* *Francisella tularensis* *Yersinia pestis*	Ebola virus Marburg virus Variola major virus (Smallpox) Variola minor virus (Alastrim)	Botulinum neurotoxins Botulinum neurotoxin producing *Clostridium* spp.

[a]Adapted from the Federal Select Agent Program website (https://www.selectagents.gov); last accessed August, 2018.

2. THE LABORATORY RESPONSE NETWORK

The LRN comprises a network of domestic and international clinical, public health, food testing, veterinary, environmental, and military laboratories that act as sentinel, reference, and national laboratories for the early detection and definitive identification of pathogens that pose significant public health threats, both those arising naturally or those intentionally released in acts of biological terrorism. The roles of each of these laboratory types are listed below.

2.1 Sentinel Laboratories

Sentinel laboratories comprise virtually all clinical laboratories within academic healthcare systems, community and military hospitals, commercial reference laboratories, and private medical laboratories. In addition, many food testing, veterinary diagnostic, agriculture,

and environmental laboratories act as sentinel laboratories. By definition, sentinel level laboratories are "certified to perform high complexity testing under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) by the Centers for Medicare and Medicaid Services (CMS) for the applicable Microbiology specialty, or the laboratory is a Department of Defense (DoD) laboratory certified under the DoD Clinical Laboratory Improvement Program (CLIP), or the laboratory is a veterinary medical diagnostic laboratory that is fully accredited by the American Association of Veterinary Laboratory Diagnostics (AAVLD)" (https://www.aphl.org/aboutAPHL/publications/Documents/Definition-Sentinel-Clinical-Laboratories.pdf). Sentinel laboratories perform in-house testing that "includes Gram stains and at least one of the following: lower respiratory tract, wound, or blood cultures". The role of these laboratories is as the name implies: they initially detect potential BT agents through routine testing of clinical, veterinary, food, and environmental specimens such as body fluids, foodstuffs, and water or soil, respectively. Of note, sentinel clinical laboratories should never test environmental, animal, food, or water samples for BT agents which have not been approved by the public health laboratory. These types of samples should be immediately directed to the nearest LRN reference laboratory. It is the responsibility of these laboratories to safely rule out microbial isolates as being BT agents through the judicious use of primarily phenotypic tests (e.g., cellular morphology, spot biochemical tests, etc.). Once a microbial isolate is suspected of being a BT agent, only the minimum number of tests required to rule out such agents must be performed to avoid generation of large-volumes of potentially dangerous subcultures. For specific examples, please refer to agent-specific ASM Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases, found at the following web address: http://www.asm.org/index.php/guidelines/sentinel-guidelines. If an isolate or isolates **cannot** be ruled out as being a BT agent, representative isolates **must** be forwarded to an LRN reference laboratory for additional testing. If further testing definitively identifies the isolate(s) as being a BT agent, it is the responsibility of the sentinel laboratory to destroy, and document the destruction of, said isolate(s) within seven days following the receipt of notification of the isolate's identification. If on-site destruction of the isolate and all testing supplies and clinical specimens linked to the agent(s) cannot be accomplished, all such material should be forwarded to a reference laboratory for proper disposal.

2.2 Reference Laboratories

LRN reference laboratories are capable of detecting biological and chemical threats including emerging infectious diseases. Tests of confirmation include additional phenotypic and genotypic (e.g., PCR) tests. These laboratories are also charged with the tasks involved in enacting a timely local response, including initiating epidemiological investigations and providing instructive feedback to sentinel laboratories, to any suspected biothreat incidents.

2.3 National Laboratories

LRN national laboratories include designated governmental public health (e.g., CDC) and military (e.g., United States Army Medical Research Institute of Infectious Diseases [USAMRIID]) laboratories that are uniquely capable of performing in-depth characterization of BT agent strains through the use of highly complex laboratory testing methods. In addition, CDC

oversees and facilitates the activities performed by reference and sentinel laboratories in local responses to BT incidents.

3. LABORATORY RISK ASSESSMENT

Risk assessments are crucial steps for laboratory biosafety. Safety risk assessments are multifaceted, ongoing processes with the ultimate goal of mitigating adverse events such as laboratory-acquired infections or release of potentially infectious agents into the environment. Laboratory safety risk assessments are different processes than Individualized Quality Control Plan (IQCP) quality control procedures. The safety risk assessment process is composed of an initial assessment of risk which considers potential laboratory hazards, existing procedural and engineering controls to mitigate exposure, evidence to support current practices, additional mitigation strategies, and documentation of findings.

Risk assessments should be performed when bringing a new assay or test process on board, when a new instrument is placed, when new laboratory staff begin working, or if a new threat or hazard is identified. For example, if a novel influenza virus is identified and is reaching epidemic or pandemic levels, a risk assessment should be performed. A general, standardized approach to each of the specific risk assessment steps is presented in the following sections. However, each laboratory must develop an individualized assessment and mitigation plan appropriate for their specific laboratory needs. It is important to note that risk assessments are a continual process that must be periodically reviewed and evaluated.
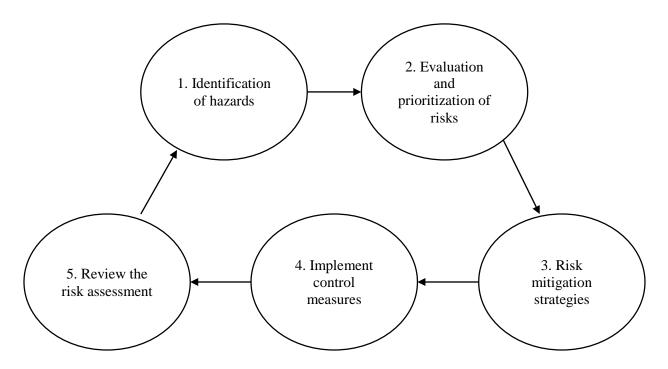

Figure 1. The risk assessment process described in the text.

3.1 How to Conduct a Laboratory Risk Assessment

Risk assessments include the identification and assessment of specific risks. Risk consists of the biological agent(s), likelihood or incidence of encountering this agent, and laboratory equipment or practices that may be sub-optimal in reducing laboratory or environmental exposure. Importantly, the assessment of risk may change depending on staff changes (such as new hires), facility and test menu changes, recognized outbreaks or biological terrorism events, and the types of samples that may harbor the agent. This in turn can affect standard laboratory practices or result in the implementation of special practices until the heightened risk is alleviated. Examples of events that heightened risk and resulted in adoption of special practices include the US anthrax attacks in 2001, the 2009 H1N1 pandemic influenza (H1N1pdm09) virus, and the West African Ebola outbreak in 2014-16. These events forced laboratories to conduct risk assessments and develop specialized protocols, based on current evidence, to mitigate risk associated with these pathogens. The overarching goal of risk assessments are to guide the implementation of mitigation strategies that are stringent enough to significantly reduce the risk of laboratory acquired infections without overburdening the laboratory and technologists with safety precautions that interfere with routine workflow and are difficult to consistently adhere to. Maintaining this balance is key to the sustainability of a safe laboratory environment. There are several ways that risk assessments can be conducted. One such proposal for conducting a full risk assessment is shown in Figure 1: 1) identification of hazards, 2) evaluation and prioritization of risks, 3) risk mitigation strategies, 4) implement control measures, and 5) review the risk assessment (1).

3.1.1 Step 1 – Identification of Hazards

The first step in risk assessment is identification of biological and procedural hazards that present increased risk. One method to identify biological hazards is to utilize established classification schemes such as the WHO and ABSA "risk group" categorization or the HHS tiered system to identify the agents most likely to pose significant risk to human health and be used in a biological terrorism attack (see Chapter 1, *Introduction*"). These classification schemes can be a useful starting point, but may not consider route of transmission or differences in relative risk between specimens, pure cultures, or growth phases of the microorganisms. Therefore, there may not be a direct correlation between a specific risk group and a corresponding biosafety level. Given these limitations, risk group or tier designation should not be the primary focus of risk assessment.

Individual laboratories should consider the most likely route(s) of infection as well as the infective form and infective dose of biological agents in their risk assessment. For example, *B. anthracis* is classified as risk-group 2 by ABSA and as a Tier 1 select agent by HHS. Patient specimens and cultures of *B. anthracis* can be safely handled using biosafety level-2 (BSL-2; see section 4, "**Sentinel Laboratory Biosafety**") precautions unless high concentrations are used or aerosols are produced. This is because the infective *B. anthracis* endospores are formed only under specific environmental conditions such as nutrient limitation, and are not typically present in clinical specimens or cultures (2). In contrast, laboratories that perform procedures that create aerosols, use high concentrations, or routinely handle environmental or soil specimens may consider the use of BSL-3 precautions for primary *B. anthracis* specimen processing because of the increased risk of endospores in these specimens. *F. tularensis* is designated as a risk-group 2

agent and is a Tier 1 select agent, and *Brucella* spp. are risk-group 3 organisms that are not Tier 1 select agents. Like *B. anthracis,* clinical specimens containing these organisms can be safely handled using BSL-2 precautions. If *F. tularensis* or *Brucella* spp. are suspected in a patient specimen BSL-3 practices should be used. Pure cultures, which have very high concentrations of organisms compared to clinical specimens, of either *F. tularensis* or *Brucella* spp. must be handled under BSL-3 conditions because of the high risk of aerosol transmission and low infective dose via inhalation (2). Specimens containing agents of viral hemorrhagic fever should be handled only under BSL-3 precautions and pure cultures should not be attempted outside of a BSL-4 laboratory.

Another consideration in the identification of biological hazards is the frequency of encountering these agents. This can be dependent on the region of endemicity for each agent, risk factors for the population served by the laboratory (e.g. foreign travelers, military, specific lifestyle or vocational risks associated with a specific pathogen), the type of specimen processed (e.g. human, veterinary, environmental), and the historical rate of identification of these agents at a given laboratory or institution. Recognized outbreaks or bioterrorism events may also increase the likelihood of encountering specific agents and should be considered when assessing risk. Procedural risks are those risks that are inherent to standard laboratory procedures used in the processing of specimens or cultures and include administrative, procedural, and mechanical features. Administrative features largely refer to the written policies and procedures for the safe manipulation of specimens and cultures in the laboratory. A lack of written policies for the handling of specimens or cultures containing hazardous organisms would constitute an administrative risk. Likewise, outdated policies that do not include current laboratory equipment and safe work practices are administrative hazards. Finally, it is critical that all staff are familiar with the policies and how to quickly access paper or electronic versions when needed.

Procedural factors encompass the adherence to universal precautions and the use of PPE appropriate for a given laboratory task (see section 4.7, "**Routes of Agent Transmission**" and section 4.8, "**Safe Handling of Clinical Specimens in the Clinical and Public Health Microbiology Laboratory**"). Good examples of procedural risks are the use of sharps (e.g. needles, razors), manipulation of primary specimens outside of a BSC, and the conduct of aerosol-generating procedures during specimen processing or isolate identification. It is important to recognize that many of these tasks are unavoidable; however, recognition of procedures that carry added risk enables the development of specific mitigation strategies to reduce the associated risk to an acceptable level. A regular survey of the laboratory noting practices not in accordance with safety policies can be a good method to identify procedural hazards. Common findings may include failure to use appropriate respiratory PPE or face shields when conducting aerosol-generating procedures outside of a BSC or use of overfilled sharps containers.

Finally, mechanical hazards include all laboratory instrumentation, including centrifuges, pipettors, automated identification systems, and BSCs. Many risks are unique to the instrument itself, therefore each piece of equipment will require an independent assessment of risk. Common risks associated with specific laboratory instrumentation are discussed elsewhere in this guideline (see section 4.8, "**Safe Handling of Clinical Specimens in the Clinical and Public Health Microbiology Laboratory**"); however, some general hazards apply to all instrumentation. A review of routine preventative maintenance specified by the manufacturer and monthly inspection for broken or non-functioning instrumentation can identify these hazards. Common hazards may include cracked centrifuge lids, dirty exhaust filters, or overcrowding of

BSCs with laboratory equipment that interferes with efficient laminar flow. For equipment essential to safety, daily or weekly function checks using airflow gages, thermometers, or tests of audible alarm systems can help identify unrecognized hazards.

A practical approach to identifying procedural risks is to follow a specimen from receipt in the laboratory through final reporting to identify all areas of the laboratory where the specimen or culture will be manipulated, and what instrumentation will be involved. This will likely require several specimens since workflow for a wound specimen being cultured is likely to be different than that of a respiratory specimen being tested by PCR. Assessment of personnel is another factor that should be considered while identifying hazards. Specifically, the laboratory workers' competency and level of experience are important factors that contribute to overall risk. For guidance regarding laboratorian competency, refer to the CDC Morbidity and Mortality Weekly Report (MMWR) article "Competency Guidelines for Public Health Laboratory Professionals" found at the following web address: https://www.cdc.gov/mmwr/pdf/other/su6401.pdf. Less experienced technologists, or those working less than full time may not be able to easily recognize unsafe work practices or faulty equipment. Conversely, even experienced technologists may fail to recognize potential hazards if they are overburdened. Laboratory technologists should be competency assessed for performance and adherence to biosafety practices. Training needs for laboratory personnel can be identified during this part of the risk assessment. Biological factors such as pregnancy or immune compromise may put specific technologists at a higher risk of certain laboratory acquired infections. While HIPPA or other regulations may preclude the employer from obtaining this information, clear communication should be made available to all staff acknowledging these risks and directing them to the appropriate resource (e.g. occupational health office) for additional information or work restriction recommendations. Vaccination or exposure status of personnel should also be considered. A record of vaccination or exposure to hepatitis B, *N. meningitidis*, or other common laboratory acquired infections and annual monitoring for seroconversion for *M. tuberculosis* can identify individuals with increased or decreased risk for these infections.

3.1.2 Step 2 – Evaluation and Prioritization of Risks

Evaluation and prioritization of hazards identified during the risk assessment enables appropriate allocation of resources (material, time, and labor) toward risk mitigation. There is no single model that that will work for every laboratory, however a weighted, multifactorial risk model will often provide the best guidance when evaluating risk. This approach assesses two key factors for each identified hazard: 1) frequency or likelihood of occurrence, and 2) severity of consequences. Each of these two factors is sub-divided into relative risk categories, which together enables assignment of the overall risk or priority for each identified hazard.

As an example, likelihood of occurrence could be stratified into rare, unlikely, possible, likely, and highly likely . The specific criteria for each subcategory could be based on the relative occurrence of each hazard using historic data, or could correspond to the expected occurrence over a fixed timeframe such as daily, weekly, monthly, and annually. An example of likelihood of occurrence is presented in Table 2.

Table 2. Likelihood of occurrence example

Likelihood	Relative occurrence
Rare	Almost never occurs; will only occur in exceptional circumstances
Unlikely	Not likely to occur in the foreseeable future
Possible	May occur within the foreseeable future. Sporadic exposure could occur.
Likely	Likely to occur within the foreseeable future. Routine exposure is likely.
Highly likely	Almost certain to occur within the foreseeable future. Consistent exposure is highly likely.

Likewise, severity of consequence could be stratified by outcome of infection such as insignificant, minor, moderate, major, and critical. The level of person to person transmission may be considered in this stratification. Some factors that impact consequence include the risk group of the organism, the infectious dose, the concentration of the agent, the environmental setting and process (for example, where the agent manipulated and what was being performed), and the host experience level and immunocompetence. An example of exposure consequence is shown in Table 3.

Table 3. Consequence of exposure example

Consequence	Outcome
Insignificant	No treatment required.
Minor	Minor injury requiring first aid treatment, or possible colonization.
Moderate	Injury that requires medical treatment or lost employee time.
Major	Serious injury requiring specialist medical treatment or hospitalization (infection and recovery).
Critical	Loss of life, permanent disability, or multiple serious injuries (disease and sequelae).

Once assigned a frequency and severity score, the hazard can be plotted on a risk matrix and a determination of risk such as "low", "medium", "high" and "extreme" can be made. A low risk may be determined to require no mitigation steps while medium risk may need mitigation. A high risk will require mitigation before the procedure is followed and an extreme risk will require significant control measures (or an alternate procedure). In general, a laboratory should strive to achieve "low" risk for all test and safety procedures. A risk matrix example is shown in Table 4.

As an example, manual removal of and transport of glass blood culture bottles from one room in the laboratory to a different room where the BSC is located for preparation of gram stain and culture could have many risks. Accidentally dropping a positive culture bottle containing *F. tularensis* could result in severe illness requiring medical attention; however, the occurrence may be judged to be rare based on the number of culture bottles broken annually and the frequency of cultures positive for *F. tularensis*. Therefore, the hazard of manual transport of a glass bottle from one room to another may be "medium" risk that at some point may require mitigation (such as a change to plastic bottles, if possible). In contrast, a non-functioning BSC would result in daily exposure to low and high pathogenicity organisms and could be categorized as anextreme risk that should be immediately rectified.

A successful evaluation and prioritization is dependent on initial identification of risks as discussed earlier. It is also important to note that, like identification of hazards, the evaluation of risk should be an ongoing process that can be expected to change with staffing, equipment, and changing epidemiology of infectious organisms.

Table 4. Risk matrix example

Matrix	Consequence				
	Insignificant	Minor	Moderate	Major	Critical
Rare	Low	Low	Low	Medium	Medium
Unlikely	Low	Low	Medium	Medium	High
Possible	Low	Medium	High	High	High
Likely	Low	Medium	High	High	Extreme
Highly likely	Medium	Medium	High	Extreme	Extreme

(Likelihood is the row axis label)

3.1.3 Step 3 – Risk Mitigation Strategies

Once hazards have been identified and risks prioritized, specific interventions to mitigate the risks should be developed. Strategies that can be used to reduce risk include, in order from most effective to least effective mitigation: elimination of the hazard, substitution of the hazard, engineering controls, administrative controls, and incorporating PPE. Since elimination of a particular hazard may not always be possible, substitution should be considered. For example, substituting glass tubes and blood culture bottles with plastic, if possible, reduces risk. Engineering controls include using safety equipment such as a biosafety cabinet, sharps containers, eyewashes, sealed centrifuge rotors, and use of secondary transport containers that can be used to minimize exposures from accidental drops and spills. Administrative controls are those that affect the way that the laboratory staff works. These include training, adherence to written procedures, appropriate use of workplace signs, hand washing, limiting the use of needles and sharps, minimizing aerosols, and appropriate PPE use. The use of PPE as a mitigation step is the last resort after all other mitigation steps have been taken. While PPE is effective in decreasing risk, more PPE can result in decreased dexterity and be uncomfortable for employees. If there is no feasible mitigation strategy for a risky procedure then it is advisable to not perform the procedure.

Another example is the potential hazard of aerosol exposure while pipetting respiratory specimens on the benchtop. In this case it is reasonable to review the laboratory existing policy for handling respiratory specimens, as well as any training and competency records appropriate for that activity (e.g. handling of primary specimens, setting up a molecular test for respiratory viruses etc.). If this activity is not specifically addressed in a lab policy, or the technologist(s) was not trained or competency assessed, the mitigation strategy may be administrative including education, re-education, or modification of a written policy. If specific engineering controls (e.g. face shields, plexiglass shields) are not adequate or available, purchase of these devices or implementation of a global policy for processing all primary specimens in a BSC could be implemented.

In some cases, a single mitigation step can reduce multiple identified hazards. Installation of "clean" sinks for handwashing at each entry point to the lab and clearly demarcating "clean" from "dirty" areas can reduce the risk of transmission of infectious agents via secondary contact with fomites in an area outside of the lab. This may also reduce the

chance of ingestion resulting from storage of food or drink in a "dirty" area. Distribution of workload among multiple technologists (if possible) or frequent rotation of laboratory duties (e.g. specimens processing, bacterial culture workup, susceptibility testing, molecular testing) can reduce hazards related to fatigue or boredom and helps maintain competency and familiarity with safe practices in each section of the lab. Implementation of weekly communications to lab personnel that highlight specific safety topics or short quizzes can identify deficiencies in understanding of safe work practices. These areas can then be the focus of reeducation efforts.

Hazards related to specific instrumentation may be mitigated by documentation of acceptable function checks. In many cases the manufacturer or a third party certified to inspect specific laboratory equipment can ensure that instruments are serviced and are functioning properly. In addition, a monthly inspection for signs of physical deterioration (e.g. cracked centrifuge cup lids, dirty air filters) by laboratory personnel can identify potential hazards before they become real hazards. Specifically, critical functions (e.g. airflow rate, instrument alarms, etc.) should be checked and results documented regularly so any out of control values are immediately recognized to prevent widespread exposure events. Finally, up to date instrument manuals and service contacts should be available in the event of malfunction. The relative infrequency of highly infectious agents is an omnipresent hazard that is difficult to mitigate. In some cases, laboratories have special handling or referral policies for these agents; however, these policies are useless if the agent is unrecognized. Therefore, healthcare institutions should establish policies requiring that members of the patient care team must communicate their suspicions of the involvement of a BT agent in a patient's disease process to the laboratory *prior* to submission of clinical specimens. Likewise, laboratories should develop a plan for communication of CDC or other health alert network advisories to bench level technologists to increase awareness of the potential for encountering agents during sporadic outbreaks or epidemics. Additionally, standard practices such as the use of BSCs and adherence to biosafety level 1-4 precautions as appropriate throughout the lab will reduce risk of exposure events. Last, part of the mitigation strategy may include developing new training programs.

3.1.4 Step 4 – Implement Control Measures

Once risk mitigation strategies have been developed then the control measures should be implemented. The control plan should be documented and clearly communicated to laboratory staff. Implementation of controls includes ensuring that laboratory workers follow standing operating procedures for all tests that are performed and for all safety procedures, including proper decontamination and disposal of chemicals and biological/medical waste. Implementation of controls also includes ensuring that proper PPE is available and used correctly. For example, appropriate PPE such as gloves, a laboratory coat, and face protection (if a biosafety cabinet is not available) should be used when subculturing a positive blood culture bottle. This PPE, though, should not be worn in clean areas of the laboratory such as an administrator's office.

3.1.5 Step 5 – Review the Risk Assessment

The last step of the risk assessment process is to review the overall process, determine the effectiveness of the implemented controls, and, if necessary, modify risk mitigation strategies. Remember, risk assessment is a continuous process that must be routinely reviewed, especially after any incidents, accidents, or illnesses that occur among staff. When incidents or accidents

occur, identify the causes, make changes, and perform follow-up training with staff. Ensure that everything is documented. In addition, review the risk assessment when changes to the procedure occur (new equipment or change to the procedure itself), when moving into a new facility or renovating an existing facility, if a new reagent is used in the lab, if a new infectious disease is identified, if a recurring problem is identified, and when new scientific information becomes available. Taken together, a full assessment of risk can aid in determining the relative risk of exposure and can guide the development or modification of standard practices focused to mitigate exposure to the high-risk pathogens most likely to be encountered. While risk can never be completely eliminated, it can be greatly reduced. Encourage laboratory staff to ask questions and to be involved in the risk assessment process.

4. SENTINEL LABORATORY BIOSAFETY

4.1 General Overview

Sentinel laboratory biosafety practices should be designed to mitigate risks associated with the manipulation of pathogenic microorganisms, including BT agents, isolated from clinical and non-clinical specimens alike. Most sentinel laboratories operate facilities that are categorized as BSL-2, which are appropriate for the handling of routinely encountered bacteria, fungi, parasites, and viruses. However, the manipulation of many BT agents requires biocontainment laboratories, such as BSL-3 and BSL-4, so it is the responsibility of sentinel laboratories to develop and validate procedures and protocols to be used when the isolation of suspected BT agents occurs. BSL-2 laboratories that do not have a BSL-3 facility should utilize enhanced BSL-2 practices and take extra precautions when working with a potential high risk pathogen. Ultimately, it is the responsibility of the sentinel laboratory director to ensure that these procedures and protocols are designed and implemented, and that laboratory personnel are thoroughly trained and kept competent. Summarized in the following sub-sections are explanations of laboratory BSLs, examples of biological agents that are handled in the various BSLs, as well as brief descriptions of various PPE used to handle infectious specimens and cultures, as well as other details associated with the transmission of pathogens, proper specimen and culture handling, and decontamination of laboratory work areas and biomedical wastes. Always keep in mind that a proper safety risk assessment should be performed for all laboratory procedures (see section 3, "**Laboratory Risk Assessment**").

4.2 Laboratory Biosafety Levels

Microbiological and biomedical laboratories are assigned BSLs based upon the pathogenicity, virulence, transmissibility, treatability/preventability, and occupational risks associated with microorganisms handled in the laboratory. As stated in the introduction to this Guideline, there are currently four recognized laboratory BSL designations, BSL-1 through BSL-4, that are intended for the manipulation and characterization of no- to low-risk agents, minimal- to moderate-risk agents, high-risk agents, and highest-risk agents (as noted in the Introduction, the BSL designations are different than the Risk Groups). Each BSL utilizes techniques deemed standard microbiological practices as well as BSL-specific enhancements, such as the use of specific engineering and administrative controls appropriate for the agents under study. Brief

descriptions of each BSL are detailed below. For further details, please refer to the CDC's publication *Biosafety in Microbiological and Biomedical Laboratories*, *5th Edition* (available here: https://www.cdc.gov/biosafety/publications/bmbl5/ as well as other appropriate references.

4.2.1 Biosafety Level-1 (BSL-1) Laboratories

BSL-1 laboratories and associated work practices are meant for work with microorganisms (Table 5) that are not known to consistently cause disease in healthy adult humans and that pose minimal risks to laboratory personnel and the outside environment. Work within BSL-1 laboratories should be performed by trained personnel under the supervision of competent scientists. Manipulations of microorganisms in BSL-1 laboratories can be safely performed on the open bench top; standard microbiological work practices such as those detailed below are sufficient; special work practices are rarely required. Examples of standard microbiological work practices utilized in BSL-1 laboratories include, but are not limited to:

- Prohibition of eating, drinking, gum chewing, smoking, contact lens manipulation, application of cosmetics, mouth pipetting, and storage of food stuffs.
- Utilization of work practices geared toward minimization of biological agent splashes and aerosol generation.
- Utilization of appropriate PPE such as laboratory coats, gloves, and eye/face protection when the risk of splashes or clothing/skin contamination is likely.
- Safe handling, decontamination, and disposal of sharps and other biologically contaminated waste items.
- Routine decontamination of work surfaces with appropriate disinfectant solutions following spills of biological materials and following completion of work.
- Handwashing following manipulations of microorganisms and prior to leaving the BSL-1 laboratory.
- Strict adherence to laboratory rules and regulations regarding access of the laboratory to trained personnel.

BSL-1 laboratories should be separated from public spaces (e.g., classrooms, offices, break rooms, etc.) and must have doors to control their access. In addition, BSL-1 laboratories should be outfitted with sinks for handwashing and should not be carpeted or lined with absorbent surfaces. All laboratory work surfaces and chairs must be made of non-porous, non-absorbent materials that are resistant to chemical and physical agents used during experimentation and for disinfection of those surfaces. If windows are located within BSL-1 laboratories, they must be fitted with screens to prevent the entrance of pests.

Table 5. Microorganisms that can be safely handled at BSL-1 unless otherwise noted.

Type	Examples	Notes
Bacteria	Asporogenic *Bacillus subtilis* *Escherichia coli* K12	These agents may be handled in BSL-1 laboratories by personnel using standard microbiological practices as long as they are not transformed or otherwise modified to contain virulence factor-encoding genes. For work with transformed organisms, assignment to a higher BSL may be required.
Fungi	*Saccharomyces cerevisiae*	
Protozoa	*Euglena* spp.	
Viruses	Adeno-associated virus Baculovirus	

Adapted from Appendix B-I, *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* – available at:
https://www.unh.edu/research/sites/www.unh.edu.research/files/docs/EHS/Biosafety/NIH_Guidelines_Risk_Groups.pdf

4.2.2 Biosafety Level-2 (BSL-2) Laboratories

BSL-2 laboratories and associated work practices are required for manipulations of microorganisms that pose a moderate risk to laboratory staff and to the outside environment. Microorganisms in this category (Table 6) are known to consistently cause mild to moderate infectious diseases in healthy adult humans. Work within BSL-2 laboratories should be performed by personnel trained to handle pathogens under the supervision of competent scientists. Many manipulations of microorganisms in BSL-2 laboratories can be safely performed on the open bench top (e.g., examination of routine bacteriological cultures derived from clinical specimens); however, all procedures that are likely to create aerosols or splashes of infectious material must be conducted using appropriate engineering controls. Work practices in BSL-2 laboratories incorporate all of those employed in BSL-1 laboratories plus additional practices that include, but are not limited to:

- A manual outlining biosafety policies and practices, including spill cleanup, emergency response, and post-exposure follow-up measures, must be available to all employees of the laboratory.
- Biohazard signs must be posted at all laboratory entrance points and biohazard labels must be affixed to all biomedical waste containers, incubators, refrigerators, freezers, centrifuges, and other devices or containers used for the storage, propagation, manipulation, and/or disposal of infectious materials.
- Biological safety cabinets (BSCs), splash shields, and other physical containment devices must be available for the manipulation of infectious agents when procedures likely to generate aerosols and splashes are conducted. Filtered exhaust air from BSCs can either be recirculated into the laboratory space or can be vented into dedicated plenums.
- Competency and proficiency assessments of employees engaged in work with infectious materials, including clinical specimens, must be periodically performed.
- Eyewashes must be available throughout the laboratory.

- Laboratories must provide appropriate medical surveillance (e.g., tuberculin skin testing) and offer vaccinations against agents for which vaccines are available (e.g., hepatitis B virus, *Neisseria meningitidis*, etc.).
- Vacuum lines connected to aspirators must be protected with in-line high-efficiency particulate air/arrestance (HEPA) filters and liquid disinfectant traps to minimize the risk of house-vacuum or dedicated pump contamination.

Like BSL-1 laboratories, BSL-2 laboratories should be separated from public spaces (e.g., classrooms, offices, break rooms, etc.) and must have doors to control their access and must have sinks for handwashing. Also, all laboratory work surfaces, chairs, and floors must be made of or lined with non-porous, non-absorbent materials that are resistant to chemical and physical agents used during experimentation and for disinfection of those surfaces. Ideally, laboratory windows should be sealed; however, if they can be opened to the outside, they must be fitted with screens to prevent the entrance of pests.

Table 6. Microorganisms that can be safely handled at BSL-2 unless otherwise noted.

Type	Examples	Notes
Bacteria	*Acinetobacter baumannii*	
	Aeromonas hydrophila	
	Bordetella parapertussis	
	Bordetella pertussis	
	Campylobacter jejuni	
	Chlamydia trachomatis	
	Clostridium perfringens	
	Corynebacterium diphtheria	
	Erysipelothrix rhusiopathiae	
	Escherichia coli	
	Haemophilus influenzae	
	Helicobacter pylori	
	Klebsiella pneumoniae	
	Legionella pneumophila	
	Mycoplasma pneumoniae	
	Neisseria gonorrhoeae	
	Neisseria meningitidis	Isolates causing invasive diseases (e.g., meningitis) should be handled in a BSC.
	Nocardia spp.	
	Non-tuberculous mycobacteria	
	Pseudomonas aeruginosa	
	Salmonella enterica	Enteric fever-causing isolates (e.g., *S. enterica* serovar Typh) should be handled in a BSC.
	Staphylococcus aureus	
	Streptococcus pneumoniae	
	Streptococcus pyogenes	
	Vibrio cholera	
	Yersinia enterocolitica	
Fungi	*Aspergillus fumigatus*	
	Candida albicans	
	Cryptococcus neoformans	

	Epidermophyton floccosum *Fonsecaea pedrosoi* *Fusarium oxysporum* Microsporidia *Microsporum canis* *Sporothrix schenckii* *Trichophyton mentagrophytes*	
Protozoa and Helminths	*Ancylostoma duodenale* *Babesia microti* *Cryptosporidium* spp. *Entamoeba histolytica* *Enterobius vermicularis* *Giardia intestinalis* *Loa loa* *Naegleria fowleri* *Plasmodium* spp. *Strongyloides stercoralis* *Toxoplasma gondii*	Manipulation of submitted collection devices should be performed in a BSC, as aerosolized eggs are infectious.
Viruses	Adenovirus BK polyomavirus Cytomegalovirus Herpes simplex viruses 1 and 2 Human coronaviruses NL63, OC43, 229E, and HKU1 Human cytomegalovirus Human metapneumovirus Influenza viruses Measles virus Mumps virus Norovirus Parainfluenza viruses Respiratory syncytial virus Rubella virus Varicella-zoster virus Zika virus	Includes all types of human adenoviruses. Excluding highly pathogenic strains of influenza A viruses (e.g., reconstructed 1918 H1N1, H5N1, H7N9, etc.), which should be handled at BSL-3 or higher.

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https://www.unh.edu/research/sites/www.unh.edu.research/files/docs/EHS/Biosafety/NIH_Guidelines_Risk_Groups.pdf

4.2.3 Biosafety Level-3 (BSL-3) Laboratories

BSL-3, or biocontainment, laboratories and associated work practices are essential for manipulations of indigenous and exotic infectious agents that pose a serious risk to laboratory personnel and to the outside environment. The agents worked with at BSL-3 are well-established pathogens that cause serious and often debilitating or fatal diseases in laboratory personnel. Many of the agents that require BSL-3 containment can be transmitted to personnel through inhalation, so facility and PPE enhancements are required to mitigate the risks of laboratory-

acquired infection. For some agents, effective therapies and vaccinations are available, but for the vast majority of viruses worked with in BSL-3 containment, no specific treatments or prophylactics are available. Agents requiring BSL-3 containment include numerous bacteria, fungi, and viruses, including many select agents; relevant examples are presented in Table 7.

In addition to standard microbiological work practices and the specialized precautionary measures utilized at BSL-2, BSL-3 laboratories and work practices incorporate numerous enhancements that include, but are not limited to:

- Incorporation of lockable, self-closing doors between the containment area and an anteroom and between the anteroom and areas outside of the laboratory suite.
- Sealing of floors, walls, and ceilings to create a single, seamless surface that envelops the containment area. In addition, any windows within the biocontainment suite must be sealed.
- Laboratory floors, walls, and ceilings must be constructed of non-absorbent materials that are finished to create smooth surfaces that are easily cleaned and decontaminated.
- Unidirectional, single-pass airflow that travels from areas of low risk to areas where high-risk work is performed. Negative air pressure is maintained within BSL-3 laboratories and associated anterooms or staging areas by way of dedicated ventilation units that draw air from workspaces through a HEPA filter prior to discharge into the atmosphere. Plenums carrying contaminated air must be sealed and must be equipped with dampers to arrest and contain the flow of air in the event of ventilation system failure. Depending upon the nature of the pathogens being studied, the inclusion of redundant HEPA filters may be required to absolutely ensure sterilization of exhausted laboratory air. The use of visual air pressure indicators and monitors is also required.
- Performance of all manipulations involving infectious substances within a Class II or Class III BSCs: no work with infectious agents is permitted on the open bench.
- Personnel must be thoroughly trained in BSL-3 work practices and must undergo periodic competency and performance assessments.
- Personnel must wear solid-front gowns, smocks, or jumpsuits (e.g., Tyvek body suits and disposable gloves). Based upon the risk assessment of the agents being studied and the manipulations being performed, respiratory protection, eye protection, disposable shoe covers, and other PPE should be worn, especially if the possibility of splashes exists.
- All wastes should be decontaminated prior to removal from the containment space. This is commonly accomplished by the use of an autoclave, such as a pass-through autoclave, but chemical disinfection or decontamination by other methods may also be appropriate.
- All facility safety features, including air-handling systems, autoclaves, and BSCs, must be certified/verified prior to opening of a BSL-3 laboratory and re-certification must occur at least annually thereafter.

As with BSL-1 and BSL-2 laboratories, BSL-3 laboratories should be separated from public spaces (e.g., classrooms, offices, break rooms, etc.) and access to these spaces must be strictly controlled. Work involving select agents requires additional regulatory measures, including strict control and documentation of agents during use, storage, and disposal. Examples of select agent-specific administrative controls include select agent inventory control. In addition, all laboratory accidents involving select agents (e.g., release of a select agent or exposure of personnel to these pathogens) must be promptly documented and reported to

institutional and public health authorities (e.g., state health departments, CDC, etc.). For reporting to public health authorities, the Animal and Plant Health Inspection Service (APHIS)/CDC Form 3 (Report of Theft, Loss, or Release of Select Agents and Toxins) **must** be completed and submitted **immediately** following an incident. For more information on the APHIS/CDC Form 3, and to get access to the form and reporting information, please refer to https://www.selectagents.gov/form3.html.

Table 7. Microorganisms that must be handled at BSL-3 unless otherwise noted. Excludes attenuated strains of all organisms listed, if applicable.

Type	Examples	Notes
Bacteria	*Brucella* spp. *Burkholderia mallei* *Burkholderia pseudomallei* *Coxiella burnetii* *Francisella tularensis* *Mycobacterium tuberculosis* complex organisms *Orientia tsutsugamushi* *Rickettsia* spp. *Yersinia pestis*	
Fungi	*Blastomyces dermatitidis* *Coccidioides* spp. *Histoplasma capsulatum*	Sporulating mould-forms of these organisms should be handled at BSL-3.
Viruses	Chikungunya virus	Except the vaccine strain 181/25, which can be handled at BSL-2.
	Eastern equine encephalitis virus	
	Flexal virus	
	Hantaviruses	Prospect Hill, Thottapalayam, and Tula viruses can be worked with at BSL-2. Work with naturally or experimentally infected animals should be performed at BSL-4.
	Japanese encephalitis virus	Except vaccine strain 14-14-2, which can be handled at BSL-2.
	Middle East respiratory syndrome coronavirus	
	Rift Valley fever virus	Except vaccine strain MP-12, which can be handled at BSL-2.
	Severe acute respiratory syndrome coronavirus	
	West Nile virus	
	Yellow fever virus	Except vaccine strain 17D, which can be can handled at BSL-2.

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4.2.4 Biosafety Level-4 (BSL-4) Laboratories

BSL-4, or maximum biocontainment, laboratories are reserved for work involving pathogens that are readily transmissible to laboratory personnel, pose significant public health threats if released into the outside environment, and cause serious and often life-threatening diseases in those they infect. To date, all known agents requiring BSL-4 containment are viruses; relevant examples are listed in Table 8. In the U.S., only a handful of such laboratories exist and most are federal government-run facilities, but a few are operated by academic institutions and one is privately owned. BSL-4 facilities incorporate all features of BSLs-1 through -3 laboratories plus several additional features, examples of which are listed below.

- Laboratory facilities must occupy dedicated spaces that are furnished with dedicated air supplies, laboratory ventilation systems, solid and liquid waste decontamination systems, building automation systems, security features, and other required systems.
- Laboratory workers are highly trained to work with RG-4 pathogens and must undergo medical surveillance and appropriate clearance screenings (e.g., background checks, etc.) prior to employment.
- Laboratory suites are physically contained within an air- and liquid-tight envelope surrounded by a buffer corridor that is also under negative air pressure. Many BSL-4 laboratories are designed to be "buildings within buildings," that is, they are autonomous structures built into the surrounding building infrastructure.
- Laboratory walls, floors, and ceilings are specially constructed and surfaced to prevent the absorption or escape of agents.
- In "suit laboratories," personnel remove all street clothing, including undergarments and jewelry, and dress in long-sleeve scrubs to which socks and 1 or 2 pairs of disposable examination gloves are taped. Following donning of these garments, researchers don positive-pressure personnel suits ("space suits") into which HEPA-filtered and conditioned air is pumped through detachable air hoses emanating from a supply-air distribution system located on the ceilings within the laboratories. Following completion of work, suits are decontaminated in a chemical shower prior to removal. Personnel must next remove scrubs, socks, and under-gloves and pass through a body shower prior to donning street clothes and exiting the facility.
- In "cabinet laboratories," personnel remove street clothing and don scrubs, gloves, and socks, as mentioned above, but, rather than donning protective suits, researchers perform manipulations of infectious substances using Class III BSCs.
- Air pressure-resistant (APR), magnetically lockable steel doors that are lined around the door perimeter with gas-inflatable bladders separate individual rooms within the suite. Prior to entry into suit laboratories, suited personnel pass through an APR door into an anteroom/chemical shower that is sealed prior to passing through a second APR door into the laboratory BSL-4 laboratory or BSL-4 laboratory suite.
- Air-locks/decontamination rooms that are connected directly to the buffer corridor should be available for the decontamination of large equipment (e.g., freezers, incubators, etc.) that are to be removed from the maximum containment area.
- All solid wastes, including non-infectious wastes and laboratory animals, are autoclaved or chemically decontaminated prior to removal from the laboratory suite. Pass-through autoclaves and chemical disinfectant "dunk tanks" are used for this purpose.

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- All liquid wastes, including non-infectious wastes, are both liquid- and heat-decontaminated prior to discharge into the municipal sewage system.
- All stocks of agents are closely inventoried and are only accessible by designated personnel who have undergone extensive training and clearance for that role.
- Security monitoring, including badge- and biometric-scanners are in place to grant laboratory personnel access to BSL-4 areas.
- Video surveillance of all BSL-4 workspaces are required to ensure personnel compliance with established protocols and to visualize accidents and emergencies so that outside security personnel can alert emergency responders when necessary.

Table 8. Viruses that require BSL-4 containment and work practices for cultivation and handling of experimentally infected animals.[1, 2]

Examples	Notes
Alkhurma Crimean-Congo hemorrhagic fever virus Ebola viruses Guanarito virus Herpes B virus Junin virus Hendra virus Kyasanur Forest disease virus Lassa virus Lujo virus Machupo virus Marburg virus Nipah virus Omsk hemorrhagic fever virus Sabia virus Tickborne encephalitis virus Variola virus	Includes Ebola virus, Sudan virus, Bundibugyo virus, Taï Forest virus, and Reston virus. Except vaccine strain Candid#1, which can be safely handled at BSL-2.

Note:

[1]Clinical specimens (e.g., blood) from patients suspected or confirmed to be infected with viruses requiring BSL-4 containment for cultivation can be handled at BSL-3 if stringent work and decontamination practices are followed. Required protective measures (e.g., level of PPE required to perform tasks), work practices, and decontamination measures should be guided by a risk assessment.

[2]Following global eradication and the cessation of oral poliovirus vaccination, all polioviruses will be designated as RG-4 viruses and work with these viruses will be restricted to BSL-4. For more information, see http://www.biosafety.be/Polio/GlobalActionPlanWHO.pdf

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4.3 Engineering and Administrative Controls

Engineering controls are defined by the Centers for Disease Control and Prevention as devices that protect workers (e.g., laboratory staff) by reducing hazardous conditions or by placing a barrier between the worker and the hazard. In microbiological and biomedical laboratories, engineering controls include:

- Air pressure-resistant doors;
- Autoclaves;
- Aerosol-containment (sealed) lids on centrifuge buckets and/or rotors;
- BSCs;
- Chemical fume hoods and benchtop fume extractors;
- High-efficiency air(or particulate arrestance) filters;
- Laboratory access control systems;
- Laboratory anterooms;
- Puncture-resistant sharps containers;
- Sharps safety devices (e.g., integrated needle sheathing devices); and
- Splash shields.

Conversely, administrative controls are defined as changes in procedural practices that help mitigate workplace hazards to workers (e.g., laboratory staff). Relevant examples of administrative controls include:

- Developing and implementing thorough standard operating procedure(s);
- Developing a hazardous substance inventory;
- Implementing a hazard communication system that uses biohazard signs, labels, and tags to identify biologically contaminated and potentially infectious materials or areas; and
- Limiting access to high-hazard areas to only well-trained, dedicated personnel.

4.4 Personal Protective Equipment

The types of PPE worn by laboratory staff is dictated by both the nature of the pathogens being studied and the type of work being performed; however, a thorough risk assessment should always be used to guide the selection of task and potential organism-appropriate PPE. For routine clinical microbiology procedures such as specimen handling and bacterial culture examination, a laboratory coat and disposable gloves are generally sufficient. When manipulations that pose a splash-risk are performed, protective eyewear or a face shield should be included in the PPE wardrobe. Regardless of the PPE used, engineering controls such as a BSC should be used if procedures likely to generate infectious aerosols are performed.

In the BSL-3 laboratory, respiratory protection such as N95 respirators or powered air-purifying respirators (PAPRs) should be worn based on risk assessment to protect the laboratory staff from infectious airborne particulates. When N95 respirators are used, personnel must undergo annual respirator fit testing and individuals who wear facial hair must wear a PAPR, as facial hair precludes the formation of a tight seal between the respirator and the wearer's skin. Surgical masks and dust masks should not be worn in BSL-3 laboratories as these masks do not meet to the performance criteria required of respiratory protection devices used in biocontainment laboratories. Instead, surgical masks should be reserved for use in patient care areas to minimize the dispersion of potentially infectious droplets emanating from infected

patients. An example of this use is in hospital emergency departments where surgical masks are to be worn by patients who have signs and symptoms of influenza-like illnesses. Additional PPE, including one or two pairs of disposable gloves, disposable gowns, shoe covers, hoods, and jumpsuits may be required for work involving high-hazard agents, including all select agents assigned to RG-3. Laboratory directors may mandate that all standard "rule out and refer" procedures be performed in a BSL-3 laboratory, if available, so the choice of PPE must be reflective of the risks associated with performing those tasks. Again, a thorough risk assessment is required to help determine the most appropriate PPE.

4.5 Exposure Monitoring and Vaccinations

Exposure monitoring may be required for work involving some pathogens such as *Mycobacterium tuberculosis* and BT agents. In most institutions, annual monitoring using an interferon-γ release assay or tuberculin skin test for detection of *M. tuberculosis* exposure is often performed on clinical laboratory personnel, especially those at the highest risk for exposure such as mycobacteriology laboratory personnel. Depending upon the frequency with which BT agents are encountered, the laboratory director, risk assessment officer, and/or occupational health professionals may require serological monitoring following a potential exposure to BT agents or as part of a routine annual monitoring process for those who work with BT agents. Guidelines have been established by the CDC with regard to post-exposure monitoring of laboratory personnel who have worked with many BT agents. For example, the CDC recommends serological monitoring at 0, 6, 12, 18, and 24 weeks post-exposure for individuals exposed to *Brucella* spp. other than *B. abortus* RB51 and *B. canis* (https://www.cdc.gov/brucellosis/laboratories/risk-level.html; also: https://www.cdc.gov/brucellosis/pdf/brucellosi-reference-guide.pdf). In addition to serological-based monitoring, personnel exposed to BT agents should participate in regular (e.g., daily) health assessments (e.g., fever checks), especially if signs and symptoms of an infectious disease are noted. For specific and up-to-date information, contact local, state, and/or national public health specialists (e.g., CDC).

In addition to exposure monitoring, laboratory personnel should be offered, at no charge to them, vaccinations for vaccine-preventable infectious diseases such as hepatitis B, meningococcal disease, influenza, and others. As with PPE, a risk assessment of activities involving infectious agents should include information pertinent regarding the recommended vaccinations that personnel should be offered.

4.6 Disinfection of Laboratory Surfaces, Workspaces, and Equipment

A vital component of clinical microbiology biosafety and infection control practices is the disinfection of the laboratory work environment and the equipment used to process, incubate, and store infectious agents. Disinfection can be defined as the elimination of most or all microorganisms, excluding spores, from, on or within an abiotic surface or matrix. Most laboratories use one or more liquid chemical disinfectants to decontaminate laboratory bench tops, phones, computer keyboards, centrifuges, incubators, and a variety of other surfaces that can become contaminated with the microorganisms being manipulated in the laboratory. The choice of disinfectant(s) used should be influenced by the types of biological agents likely to be present on laboratory surfaces and the compatibility between the disinfectant(s) and the surfaces

since not all disinfectants are broadly germicidal and some may destroy certain surface materials. Regardless of the disinfectant used, the manufacturer's specifications regarding disinfectant working-stock preparation (if provided as a concentrate) and use must be followed. Deviation from these instructions could result in ineffective disinfection or other unwanted consequences, potentially leading to a laboratory-acquired infection or other negative outcome.

In general, to effect thorough decontamination, the disinfectant must be applied to a surface at the working concentration specified in the package insert or according to the directions on the container, it must remain in contact with the surface for the recommended amount of time, and the temperature of the disinfectant and the surface to be decontaminated must be within the manufacturer's allowable range so as to not inactivate the disinfectant or create harmful vapors. Other factors that influence disinfectant efficacy include the presence, type, and quantity of biological soils (e.g., blood and body fluids), the pH and relative humidity of the disinfection process and surrounding environment, and the nature of the object being disinfected. Many hospital-grade disinfectants require that blood, body fluids, feces, and other biological fluids or substances be removed prior to application of the disinfectant on the surface. This can be achieved by absorbing the soil with a disposable wipe, gelling powder, or other absorbent followed by removal and disposal of the absorbed material. Subsequently, working-strength disinfectant should be applied to the surface, allowed to remain for a suitable contact time, and then wiped up with a clean absorbent wipe.

The subsections below briefly summarize the types of chemical disinfectants used in clinical microbiology laboratories, their spectrum of activity, and the process for biological spill cleanup as well as whole-laboratory decontamination. For specific information, please refer to relevant texts such as the CDC publication *Biosafety in Microbiological and Biomedical Laboratories, 5th* edition.

4.6.1 Levels of Disinfection and Types of Chemical Disinfectants

Three levels of disinfection (low, intermediate, and high) are currently recognized by the CDC and others, and are defined by the spectrum of the germicidal activity exhibited by a disinfectant. Table 9 summarizes each level of disinfection and provides examples of disinfectants in each category. Note that contact times for disinfectants vary depending on the type of disinfectant and the type of microorganism the disinfectant is used against (https://www.cdc.gov/infectioncontrol/guidelines/disinfection/disinfection-methods/chemical.html).

Table 9. Levels of disinfection and examples of disinfectants that achieve each level.

Level	Spectrum of Activity	Examples of Disinfectants
Low	Effective against most vegetative bacteria (but not spores), some fungi, enveloped viruses and some non-enveloped viruses.	Quaternary ammonium compounds
Intermediate	Effective against all vegetative bacteria, including *Mycobacterium tuberculosis*, but not spores, most/all fungi, and all enveloped, and some non-enveloped, viruses.	Isopropanol (60 – 95% aqueous solutions) Phenolic compounds
High	Effective against all vegetative bacteria and their spores, fungi, and viruses.	Glutaraldehyde Chlorine (e.g., hypochlorite)

Table 10 summarizes a variety of chemical disinfectants as well as their spectra of activity and indicated uses. For a specific listing of Environmental Protection Agency (EPA)-registered disinfection products, please refer to the EPA website: https://www.epa.gov/pesticide-registration/selected-epa-registered-disinfectants. Readers must be aware that all disinfectant solutions have limited life spans, so all products should be used according to manufacturer specifications and before the expiration date printed on the product container. Information regarding contact time, reactivity, stability, etc. can be found in disinfectant solution package inserts and/or safety data sheets, so readers should consult such documents for more information. For 10% household bleach solutions, daily preparation is required.

Table 10. Types of chemical disinfectants.

Disinfectant Type	Examples	Spectrum of Activity	Notes
Alcohols	Ethanol Isopropanol	Intermediate-level disinfectants; effective against vegetative bacteria, fungi, and most viruses. **Not effective against bacterial spores.**	Aqueous solutions of alcohols made to a final concentration of 60 – 95% should be made for disinfection purposes. Refer to product insert or safety data sheet for working-stock stability, reactivity, contact time, and other pertinent information regarding alcohol solution use.
Chlorine	Hypochlorite solutions (e.g., household bleach)	High-level disinfectants; effective against virtually all bacteria, mycobacteria, fungi, and viruses. **Sporicidal activity is variable.**	Solutions of household bleach should be prepared fresh daily. Generally, a 1:10 dilution of bleach in water is made. Refer to product insert or safety data sheet for working-stock stability, reactivity, contact time, and other pertinent information regarding chlorine solution use. Generally, bleach solutions (e.g., 10% solutions) should be prepared fresh daily.
Hydrogen peroxide	Hydrogen peroxide cleaner-disinfectant wipes	High-level disinfectants; effective against virtually all bacteria, mycobacteria, fungi, and	Several products are currently available for surface disinfection. Refer to product insert or safety data sheet for

		viruses. **Sporicidal activity is variable.**	working-stock stability, reactivity, contact time, and other pertinent information regarding hydrogen peroxide solution use.
Quaternary ammonium compounds	Spray solutions and moistened wipes	Low-level disinfectants; effective against many bacteria, fungi, and enveloped viruses. **These compounds are not effective against many non-enveloped viruses and are not sporicidal.**	Refer to product insert or safety data sheet for working-stock stability, reactivity, contact time, and other pertinent information regarding quaternary ammonium compound solution use.

4.6.2 Biological Spill Cleanup and Other Relevant Topics

Biological spills consist of spills of potentially infectious liquids, including broth microbial cultures, blood and other body fluids, and liquid infectious wastes. For spill cleanup, it is essential that, immediately following the spill, all personnel surrounding the affected area are evacuated to avoid exposure. In addition, depending upon the size of the spill, the area should remain vacant until all aerosolized droplets have settled (usually 30 minutes following the spill). Warning signs should be posted in areas where a spill has occurred so that personnel are aware that it may not be safe to enter and, for spills of high-hazard agents, access to the contaminated area should be restricted to spill responders only. Prior to attempting spill cleanup, personnel must don appropriate PPE (e.g., face/eye protection, respiratory protection, gloves, disposable gown or laboratory coat, shoe covers, etc.) and assemble all needed supplies. A good practice for laboratories to adopt is the creation or purchase of biological spill cleanup kits, which should contain all necessary PPE and supplies. Following PPE donning and supply assembly, spill responders should survey the spill site for broken glass and/or other sharps; if present, sharps should be handled with tongs or forceps to avoid puncture wounds and should be discarded into a sharps container. A liquid absorption and solidification agent should next be dispersed over the spill; if not available, paper towels should be laid over the spill. If paper towels are used, they should be drenched in an appropriate disinfectant solution following spill absorption. All absorbent-disinfectants should be allowed to remain in place long enough to ensure deactivation of the pathogen(s) present. Refer to the powder/disinfectant guidelines for product use. Following absorption/solidification and disinfection, use a disposable scoop to remove and discard solids. If paper towels or another absorbent is used to absorb spills, use tongs to pick up and discard them. Next, spray a suitable disinfect over the surface and allow the compound to sit for the appropriate amount of time prior to wiping up with fresh paper towels. Finally, carefully remove and discard PPE. All wastes generated from spill cleanup should be considered biohazardous and should be disposed of with other biohazardous waste.

Spills within BSCs are not uncommon and should be dealt with in a similar fashion to spills occurring on the laboratory bench or on the laboratory floor. Some important considerations for BSC spill response include allowing the BSC blower to continue to run and immediately removing contaminated PPE followed by hand/skin washing prior to spill cleanup. Contaminated gloves should be left within the confines of the BSC workspace to avoid

contamination of items outside of the BSC. Small spills such as those in which just a few milliliters of liquid has been spilled can be immediately cleaned up using paper towels or another absorbent plus a liquid disinfectant solution. Disinfected absorbent materials should be disposed of within the biohazard bag located within the cabinet. To clean up larger volumes of spilled liquids, the same general principles as described before should be used; however, all items within the cabinet should be considered contaminated and must be cleaned and disinfected. Large volumes of infectious liquid should be absorbed with paper towels or solidified with an absorbent powder. All sharps (e.g., broken glass) should be handled with tongs or forceps and discarded in a sharps container. Follow spill cleanup, all surfaces should be again wiped with disinfectant prior to resuming work.

On occasion, entire laboratory spaces may need to undergo decontamination, including after large-volume contamination incidents and times preceding routine maintenance of a biocontainment laboratory or suite of laboratories. In the clinical microbiology laboratory, whole-laboratory decontamination may be required in the event of a large-scale release of a BT agent or other high-consequence pathogen, but, otherwise, it is rarely required. For decontamination of entire laboratories, all penetrations to the area should be sealed to facilitate surface disinfection or fumigation and cleaning. For fumigation of laboratory spaces, chemical agents such as formaldehyde gas, peracetic acid, hydrogen peroxide vapor, and chlorine dioxide are most commonly used.

4.7 Routes of Agent Transmission

The initial risk factor for laboratory acquired infections, including those caused by BT agents, is exposure to the pathogen. Common routes of entry include inhalation, ingestion, direct contact with mucosal membranes (e.g. conjunctivae, oral, nasal), and through microabrasions or other open cutaneous lesions. Percutaneous exposure through accidental needle stick is a comparatively rare route of exposure for laboratory staff, but carries an elevated risk for transmission of viral bloodborne pathogens. Physical and biological factors specific to each microorganism dictate the common route(s) of transmission and the type of precautions required to safely work with specimens or cultures containing each organism. These factors include size, infective stage (e.g. vegetative cell versus spore, mold versus yeast phase), environmental stability (e.g. ability to resist desiccation, temperature, chemical disinfectants), the ability to be easily aerosolized, and the infective dose required to cause disease (ID_{50} or ID_{90}).

Natural infection with BT agents is a relatively rare occurrence, and is likely low on the clinical differential unless there is a known outbreak or deliberate act of bioterrorism. Specimens containing these agents are usually submitted to the laboratory without notification and are handled per routine laboratory processes until a BT agent is suspected based on gram stain, culture, or other laboratory result. Therefore, it is important to maintain standard safe laboratory practices appropriate for the specimen type to reduce the risk of laboratory acquired infection. These practices fall into three main categories; contact and bloodborne, droplet, and aerosol precautions. The organisms, work practices, and risk of exposure associated with these routes are discussed in the following sections.

4.7.1 Contact and Bloodborne Transmission

Contact and bloodborne transmission refer to infections resulting from *direct contact* with microorganisms or clinical specimens such as blood, stool, pus, and other bodily secretions. Importantly, this also includes contact with inanimate surfaces such as laboratory benchtops, telephone handsets, or computer keyboards which can be contaminated with viable microorganisms. Bacteria that form endospores such as *Bacillus* spp. (including *B. anthracis*) and *Clostridium* spp. have the greatest ability to persist in the environment and be transmitted indirectly. Non-enveloped viruses such as norovirus and enterovirus are also environmentally stable and are commonly associated with contact transmission from fomites. Vegetative bacteria including *Staphylococcus* spp., *Acinetobacter* spp., *Enterococcus* spp. and *Pseudomonas* spp. are less persistent but due to their ubiquitous nature environmental contact transmission of these organisms is common as well. Bloodborne pathogens are often labile outside a host and require direct contact between the infected fluid and mucous membranes or percutaneous inoculation to initiate infection. Common examples include HIV, hepatitis B and C, and agents of viral hemorrhagic fever (e.g. Ebola, and Marburg).

Many BT and emerging infectious agents can be transmitted through contact exposure; however, this may not be the most efficient route of transmission and often results in slowly progressing or less severe disease. For example, transmission of *F. tularensis* through contact with an open wound or microabrasion results in an ulceroglandular presentation characterized by an indolent course and limited dissemination to regional lymph nodes. This form of tularemia may cause significant and protracted morbidity but is rarely fatal, and in some cases, resolves without antibiotic treatment (3-5). Similarly, cutaneous exposure to *B. anthracis* results in localized ulcerative lesions which can be severe; however, these infections only rarely disseminate and carry a mortality rate of <1% if treated appropriately with antibiotics (6). The risk of infection with bloodborne pathogens following percutaneous exposure varies widely, ranging from as high as 30% for HBV, to 3% for HCV, and 0.3% for HIV (7). Among bloodborne BT agents such as Ebola, Marburg, and Lassa virus, specific transmission rates are not well established; however, the infective dose may be as low as 1-10 virions which undoubtedly contributes to the high rate of acquisition among healthcare workers and family members having direct contact with infected individuals (2). Transmission of these viruses via fomites does not appear to be a significant risk in the setting of natural outbreaks, but may be greater in laboratory settings (8, 9). Specifically, Ebola virus persists for longer periods on surfaces when environmental factors such as temperature and relative humidity are controlled, and also has a longer half-life on surface materials such as Tyvek which are more common in laboratories (9).

Standard (universal) precautions denote the basic practices that should be employed to prevent contact or bloodborne transmission of pathogens within the hospital and clinical laboratory. It is important to note that biological safety procedures performed in hospital and clinical laboratories are different than hospital infection control guidelines. In essence, standard (universal) precautions in hospital and clinical laboratories stipulate that all human specimens (respiratory, blood, tissue, stool, etc.) be treated as if they contain infectious pathogens. Implementation of standard (universal) precautions involve both procedural and engineering controls (discussed in detail in "*Engineering and Procedural Controls*" section), as well as general safe laboratory practices. The cornerstone of standard precautions is good hand hygiene practices. Additional components of standard precautions may vary based on the risk assessment

associated with the specimen type and laboratory procedures being conducted, but may include the use of gloves, face shields, or respiratory protection. Adherence to these precautions is effective in reducing the risk of direct contact transmission of infectious microorganisms including BT agents, and reduces the risk of transmission of pathogens from unrecognized sources such as fomites.

Safe laboratory practices are a procedural control that reduce contact transmission and serve as the basis for preventing laboratory acquired infections. Clearly defining and demarcating "clean" and "dirty" areas within a laboratory with physical barriers (walls, doors), signage, or other visual cues aids in alerting employees and visitors to locations where standard precautions need to be observed. Hand hygiene practices should be rigorously followed whenever the is a recognized exposure to clinical material and before exiting the laboratory. Proper hand washing technique consists of at least 20 seconds of scrubbing all surfaces of hands using soap and clean water. Some experimental data have suggested the use of soap containing antimicrobial agents such as triclosan or chlorhexidine gluconate may be superior to standard soap and alcohol gels, reducing bacterial counts on the hands by 70-99% depending on the antimicrobial and frequency of use (10). However, in 2017 the US FDA banned manufacurers from adding antibacterial agents such as triclosan to over the counter soap products citing a lack of objective evidence that these agents provided added benefit over thorough hand washing using plain soap and water. Eating, drinking, or storing food products in the laboratory should be forbidden because of the risk of direct contact with contaminated surfaces, including unwashed hands. Similarly, the use of personal electronics such as digital music players or cellular phones should be restricted. These are "high touch" items that can become easily contaminated by soiled hands or gloves during routine laboratory work and have the potential to spread microorganisms outside of the laboratory.

PPE appropriate for prevention of contact transmission include gloves and laboratory coat. Disposable gloves reduce the risk of unrecognized contact transmission when manipulating specimens and should always be worn when handling primary specimen containers. Gloves should be made of material appropriate for the task being performed. For example, latex gloves are adequate for incidental contact with clinical specimens but are not sufficient for handling of solvents. Additionally, punctures may be difficult to detect and latex can cause allergic reaction in some people. For these reasons, many laboratories use nitrile gloves which are non-allergenic and better withstand contact with solvents. When using disposable gloves, it is good practice to check for rips or punctures before use. Gloves should be removed and changed if visibly soiled and prior to touching objects such as keyboards, phone receivers, door knobs, or water faucets. Dedicated laboratory coats provide an important physical barrier between the laboratory environment and personal "street" clothing or scrubs, which are worn outside the laboratory. Laboratory coats should be impervious to liquids and ideally be snug fitting at both the wrist and collar to provide the maximal level of protection. This barrier can be especially important in the event of unexpected splashes or spills involving liquid specimens.

Accidental needle stick or other sharps injury can present the highest risk of laboratory acquired infection through direct inoculation of microorganisms to sterile sites including the bloodstream. Laboratories should have policies in place to address specimens received in syringes with the needle still attached. This may include a strict rejection policy for such specimens as well as notification of the individual, supervisor, or medical director of the hospital unit or clinic submitting the specimen. Likewise, specimens arriving in broken glass containers (e.g. blood culture bottles, isolator tubes, etc.) should be rejected because of the risk of cut or

puncture injury, but also because of the high likelihood of external contamination of the specimen. The use of blunt needles is encouraged for laboratory procedures requiring syringe transfer of liquid specimens. If standard needles are necessary, they should never be recapped and ideally should be equipped with a safety device that can be activated before discard. All sharps, including needles, broken glass, and razor blades should be discarded in hard sided, puncture resistant containers. These containers should not be filled beyond > 75% capacity. This will prevent difficulty when discarding sharps and will reduce the chance on injury when sealing the container for disposal. All potential bloodborne exposures involving sharps or contact between a specimen and mucous membrane or non-intact skin should be immediately reported and referred to an occupational health office for assessment of exposure and risk of infection.

4.7.2 Droplet Transmission

Droplet transmission pertains to pathogens acquired through direct contact between infectious droplets and the oral, nasal, or conjunctival mucosa. Most commonly, these include respiratory pathogens present in secretions generated through talking, sneezing, or coughing. Because infection requires direct contact with infectious droplets, natural droplet transmission is restricted to a zone within 3-5 feet of a contagious individual. Highly pathogenic emerging respiratory viruses such as novel strains of influenza A (e.g. avian H5N1 and H7N9) and members of the Coronavirus family (e.g. SARS-CoV and MERS-CoV) are good examples of pathogens that are transmitted by infectious droplets and have resulted in laboratory acquired infections (11, 12). Individuals presenting with the pneumonic form of plague are capable of transmitting the BT agent *Y. pestis* via respiratory droplets, though this risk is low when compared to transmission of respiratory viruses (13). Importantly, these pathogens are often sensitive to desiccation and other environmental stresses and have limited viability on surfaces. Environmental persistence of influenza is both strain and condition dependent, but typically decreases 2-5 $\log_{10}$ within 24-48 h (11, 14, 15). Coronavirus may remain viable for as little as 3 h on surfaces (11, 14). These data suggest that the risk of contact transmission may be reduced when compared to more hardy organisms; however, laboratory surfaces where specimens containing these organisms are frequently handled still present a potential source of transmission.

Many of the laboratory procedures commonly employed during initial processing or downstream manipulation of clinical specimens or cultures have the potential to generate infectious droplets. Specific examples include venting of positive blood culture broths for gram stain and culture inoculation, performance of the catalase test on culture isolates, centrifugation to concentrate specimens, vortexing of isolates to make a bacterial suspension, and the practice of "hot looping" (touching a heat sterilized inoculating loop to agar plate to speed cooling). Manual pipetting of liquid specimens (e.g. respiratory specimens in transport medium or bacterial suspensions) is another common procedure associated with the generation of droplets, as are automated identification or susceptibility test systems that involve bacterial suspensions (see section 4.8.3, "**Special Considerations – The Use of Microbial Identification Systems for High-Risk Pathogen Identification**").

Unlike natural generation of droplets through coughing or sneezing, mechanical manipulations produce droplets with larger size variation. This impacts both the settle rate and the number of infectious organisms that can be contained in each droplet. Larger droplets will typically settle faster and have a narrower zone of transmission, but can carry a larger number of

microorganisms. Conversely, smaller droplets (referred to as aerosol or micronuclei) may take longer to settle which increases the range of transmission beyond the generally accepted 3-5-foot zone. This puts a larger proportion of the laboratory and more laboratory staff at risk of infection, especially when considering microorganisms such as *Brucella* spp., *F. tularensis*, and *C. burnetii* that have an infectious dose of 10-100 viable cells. In a review of laboratory acquired *Brucella* spp. infections >90% were associated with infectious droplets or aerosols generated during routine culture manipulations (16). The attack rate was 40-60% for persons working directly with cultured bacteria; however, 20% of labortorys staff without direct contact also acquired brucellosis. The highest attack rates were observed when working with a culture outside of a BSC, often prior to recognition of the organism as *Brucella* spp. (16). Though comparatively rare, *F. tularensis* has been reported as the second or third most common cause of laboratory acquired infection among BT agents (17, 18). Like *Brucella* spp., laboratory transmission is primarily via aerosol with the conduct of aerosol-generating procedures outside a BSC carrying the highest risk of infection. Because of similarities in growth rate, requirement for specific nutrients, and gram stain morphology, *F. tularensis* is readily mistaken for *H. influenzae* and cultures are worked up outside of a BSC leading to exposure events (19). These examples underscore the importance to maintain vigilance for potential BT agents and safe work practices regardless of specimen type.

In light of the recent outbreak of Ebola virus in Western Africa, much attention has focused on the route of transmission to healthcare workers treating these patients, as well as to laboratory workers that handle clinical specimens. Blood borne and direct contact transmission via bodily fluids is associated with a high attack rate and can carry a mortality rate of up to 90% (2). The risk of droplet transmission is likely dependent on the stage of infection (viral load in bodily secretions is highest during the acute phase of infection) and presence of clinical symptoms such as severe diarrhea, vomiting, and severe coughing, all of which can generate infectious droplets. Healthcare workers caring for patients are likely at a higher risk of infection due to the uncontrolled and unpredictable nature of the environment and patient, as well as the medical procedure that may be necessary to care for these patients such as ventilation, mechanical resuscitation, and placement of intravenous catheters. Several cases of laboratory acquired Ebola virus infections have been reported, but these have been restricted to direct percutaneous exposure, primarily in research laboratories (20). As of the writing of this guideline, a search of the NCBI PubMed database (search term: Ebola laboratory acquired infections) revealed zero reports of Ebola infection acquired during the handling or processing of clinical specimens collected from a symptomatic patient.

Prevention of laboratory acquired infections due to droplet or aerosol transmission relies on engineering and procedural controls, including PPE. The most effective approach to preventing droplet or aerosol transmission is to handle specimens and cultures within a BSC. Both class I and class II BSCs are open front cabinets that allow easy access to specimens and cultures but also provide protection against the release of droplets and aerosols using laminar air flow. In both types of BSCs, air is exhausted through a high efficiency particulate air (HEPA) filter before being vented externally or recirculated into the laboratory. The primary advantage of a class II BSC is pre-filtration of the air used for laminar flow which additionally reduces the risk of external contamination of specimens. The use of sealed rotor centrifuges is another engineering control that should be used to mitigate the release of infectious aerosols. If specimens are known to contain BT agents or there is visual evidence of a broken tube regardless

of the organism, the rotor should be transported to a BSC prior to opening and should be thoroughly disinfected within the BSC before being put back in to service.

For most laboratories, conducting all culture workup within a BSC is impractical. When working outside a BSC, additional protection for face and eyes should be used when conducting procedures with a risk of droplet generation. In general, this includes work with any liquid specimen (e.g. swab specimens in viral transport medium, bacterial suspension) or procedure that can easily result in spills or splashes (e.g. vortexing, centrifugation, pipetting). The specific PPE appropriate to reduce droplet transmission includes a combination of surgical mask and goggles or a face shield to provide a barrier and prevent droplet contact with mucous membranes in the mouth, nose, and eyes. Since droplets are relatively large, the use of surgical or other non-N-95 type masks are acceptable to prevent transmission. Working with specimens behind a clear Plexiglas shield on the benchtop also provides a barrier to droplet transmission; however, this approach may create a false sense of security since small aerosols may extend beyond the edges of the barrier. Again, if a BT agent suspected, no further work should be conducted outside of a BSC.

Other general procedural controls can further minimize the risk of aerosol exposure when working with infectious specimens. The use of an absorbent pad to cover surfaces where pipetting occurs (both inside and outside of a BSC) reduces aerosols resulting from drips or small spills by rapidly absorbing the fluid rather than generating a microdroplets when drips hit a hard surface. Tubes with snap-type lids should be avoided in favor of threaded screw-on caps to reduce creation of droplets when these containers or opened. Further, gauze pads can be used when opening specimen containers to mitigate aerosols released by surface tension bubbles at the mouth of the container. When using a manual pipette, the retention volume should not be expelled since this can be a source of aerosol. If pipetting samples containing a BT agent, disinfectant can be aspirated into the pipette tip immediately after dispensing the infectious sample and before ejecting the pipet tip into the waste. This will ensure contact between the agent and disinfectant and reduce the risk of infectious aerosols when ejecting the pipette tip.

4.7.3 Airborne Transmission

Airborne transmission differs from aerosol or droplet transmission in that the infectious agents do not require a liquid substrate (e.g. respiratory secretions) to aid in transmission or maintain viability. Microorganisms associated with airborne transmission are typically small, ranging from 1-10 µm in diameter, and are resistant to environmental stresses including heat and desiccation (21). This enables transmission on air currents over long distances (>1 m) and for extended periods of time. Within the hospital and laboratory this presents an added risk for widespread transmission via heating, ventilation and cooling (HVAC) systems (21). Rubeola (measles), Variola (smallpox), Varicella (chickenpox), *Mycobacterium tuberculosis*, Hantavirus, bacterial endospores (e.g. *B. anthracis, C. burnetii*) and fungal spores (e.g. *Aspergillus spp.* and *Coccidioides spp.*) are examples of microorganisms associated with airborne transmission.

The source of airborne transmission can be aerosol micronuclei, but may also be dust, skin flakes, or the naked organism itself. Some BT agents not associated with natural airborne transmission have been engineered to be disseminated as a powder or granular form that prolongs viability and extends the physical range of dissemination. Most notably, these include *B. anthracis*, *F. tularensis* and *Y. pestis*. When delivered via airborne route, inhalation of as few as 10 of these organisms can cause disease with mortality rates of 40-99% if untreated (2).

Airborne transmission of Ebola and other bloodborne agents of viral hemorrhagic fever is unlikely under natural conditions, even during epidemic outbreaks (22). However, transmission may occur through generation of micronuclei during medical procedures such as mechanical ventilation or during episodes of projectile vomiting or diarrhea.

Prevention of airborne transmission within the hospital and laboratory relies on directional airflow and physical containment specimens and cultures. Cultures containing dimorphic fungi or any other suspected BT agent should be handled exclusively in a BSC, preferably within a negative pressure suite within the laboratory (e.g. BSL-3 type laboratory). Specific components required to designate a BSL-3 laboratory are discussed in the section of this guideline titled "**Biosafety Level-3 (BSL-3) Laboratories**" (section 4.2.3), but a major component is negative pressure and directional airflow. Negative pressure is measured as the difference in pressure between two adjacent rooms, and should be > 0.01 inches of water gage or >2.5 Pascals as measured by a manometer or other device (23). If maintained, negative pressure contains airborne pathogens within the designated room and prevents exposure events within the main laboratory. The rate at which air is exchanged (exhausted and replaced with new air) within the negative pressure suite is referred to as room air changes per hour (ACH). A general relationship exists between increased number of ACH and reduced transmission of airborne pathogens (24). However, the optimal number of ACH is multifactorial involving humidity, size of the airborne particulates, infective dose, and air turbulence within the room (e.g. furniture, movement of laboratory staff, shape of room). The recommendation for laboratories handling *M. tuberculosis* and other airborne pathogens is 6-12 ACH (23, 25). If a spill occurs, sufficient time should be allowed to reduce the presence of airborne contaminants by 99% (23). This is estimated at 46 min for an ACH of 6 and 23 min for an ACH of 12 based on the equation $t_1 = [\ln (C_2/C_1) \div (Q/V)] \times 60$ where concentration of contaminants (C), airflow rate in ft^3/hr (Q), and room volume in ft^3 (V) are considered.

Within the negative pressure suite, all specimen and culture manipulations should be carried out within the BSC for primary containment. Air from BSCs in a negative pressure suite should be externally exhausted through a dedicated air duct; however, if this is not possible air may be recirculated after HEPA filtration (23). If externally exhausted, the BSC can also serve as the terminal exhaust and aid in maintaining negative room pressure; however, the BSC must be fitted with a thimble to prevent interruption of laminar flow within the cabinet in the event of reversed airflow (positive pressure). If air from the BSC is recirculated, a separate ventilation system must be in place to maintain negative room pressure. Centrifuges with sealed cups and sealed rotors should be utilized, and cups should be opened only within the BSC to contain any particulates or aerosols generated during centrifugation or opening of the specimen or culture container. If a spill occurs within the BSC, it may be cleaned up immediately and the BSC should remain on with the sash set at an appropriate level to maintain laminar flow and contain aerosols within the BSC. A paper towel soaked in disinfectant can be used to initially cover the spill and inactivate microorganisms prior to a more thorough decontamination and cleaning protocol. All waste, excluding sharps, should be discarded in sturdy leakproof plastic bags and closed prior to removal from the BSC and appropriate disposal (see section 6, "**Biomedical Waste Management**").

A risk assessment should be conducted to determine the appropriate PPE requirements. This should consider the likelihood of contact with a pathogen (e.g. working with pure culture vs. clinical specimen), the potential severity of infection in the event of accidental exposure (e.g. pathogen risk group designation), and other work practice and engineering controls that are in

place to prevent exposure. At a minimum, PPE to prevent airborne transmission must include the use of a respirator. Unlike masks recommended for prevention of droplet transmission, respirators must effectively prevent inhalation of small airborne particles. Therefore, these respirators must be tight-fitting around the nose and mouth and remove >95% of particulates ≥0.3 µm in diameter. This type of respirator is often referred to as an "N-95" respirator, and conforms to United States NIOSH standards. Importantly, these respirators should only be used after appropriate training and fit testing to ensure maximal protective benefit. Disposable N-95 respirators are adequate for relatively low risk activities such as processing of respiratory specimens or cultures suspected to contain *M. tuberculosis*. Additional respiratory protection may be warranted if working with pure cultures or specimens suspected to contain higher risk group pathogens such as Ebola, or for laboratory staff who cannot wear standard N-95 type respirators (see section 4.4, "**Personal Protective Equipment**"). In these cases, the use of a PAPR can provide additional respiratory protection equivalent to 2.5-100 times that of a standard N-95 respirator (26). An additional benefit of the PAPR is protection from contact and droplet transmission associated with skin or conjunctival exposure. Reusable PAPR hoods should be inspected for rips, punctures, or cracks prior to each use and cleaned with an approved disinfectant after each use. The functionality of the blower pack should be tested prior starting work with clinical specimens using a "floating ball" air tube to ensure a constant airflow rate of 115-170 liters/min (26). Importantly, the use of respirators and other PPE does not substitute for a non-functioning BSC or negative pressure suite in preventing airborne transmission within the laboratory.

4.8 Safe Handling of Clinical Specimens in the Clinical and Public Health Microbiology Laboratory

Laboratories should have specific policies in place for the safe handling or referral of specimens known or suspected to contain BT or other highly infectious agents. Included in this policy should be instruction for hospital or provider group(s) to notify the laboratory when diagnostic specimens are collected from patients with symptoms and/or history compatible with these agents. Unfortunately, despite the implementation of such policies exposure to BT or other highly infectious agents is an all too frequent occurrence. These exposures and resulting infections are often attributable to a low index of suspicion for these agents or failure of the hospital service to notify the lab when there is a compatible patient history and/or clinical presentation. Failure to notify the laboratory of a case of presumed acute pulmonary tularemia resulted in exposure of 11 laboratory workers to cultures of *F. tularensis* which were being worked up outside of a BSC (19). Similarly, twenty-one laboratory workers were exposed to *B. pseudomallei* because of incorrect identification of the isolate (27). These system failures serve as warning for laboratories and individual laboratory staff to remain vigilant for cultures or isolates with characteristics consistent with BT agents. It also underscores the need to implement and maintain protocols for safe work practices that pertain to all clinical specimens and cultures. Always perform a risk assessment for all procedures performed in the laboratory and remember to continually update the risk assessment over time.

4.8.1 Processing of Clinical Specimens

Clinical specimens, including swabs, washes, and bodily fluids, should always be treated as if they contain infectious agents, a practice referred to as "standard precautions." In a laboratory setting, this includes the use of a class 2 BSC and appropriate PPE in accordance with BSL-2 practices. Specifics of biosafety level requirements, appropriate PPE, and common routes of transmission are discussed elsewhere in this guideline. Specimen containers that are visibly leaking pose an increased risk of transmission, but also indicate potential external contamination of the specimen. Depending on the extent of the leak, nature of the specimen (e.g. sterile fluid vs. stool, retrievable vs. irretrievable source), and test order (e.g. culture vs nucleic acid amplification test) it may be appropriate to decontaminate the exterior container and proceed with specimen processing or to outright reject the specimen.

Microorganisms with a low infectious dose such as *Brucella* spp., *F. tularensis*, and *Y. pestis*, pose the highest risk of infection from primary specimens; however, specimens containing these organisms can still be handled safely using BSL-2 precautions (2). Exception is reserved for manipulations with a high risk of droplet or aerosol generation, in which case escalated BSL-3 precautions should be considered. Specimens suspected to contain other highly infectious BT agents or those capable of causing severe disease such as *C. burnetii*, Variola virus, and agents of viral hemorrhagic fever require BSL-3 or BSL-4 containment and should be forwarded to the appropriate LRN reference laboratory unless the clinical lab has specific protocols, training, and containment facilities to safely work with these specimens.

4.8.2 Manipulation of Microbial Cultures

Manipulation of cultures is associated with increased risk of infection when compared with handling of primary clinical specimens. This is the result of a higher concentration of organism and the conduct of aerosol-generating procedures during routine identification of culture isolates (see section 4.7, "**Routes of Agent Transmission**"). These factors contribute to the increased relative risk of infection among laboratory workers, ranging 8.6 for *E. coli* O157:H7 to 40.8 for *N. meningitidis* and 8,012 for *Brucella spp.* when compared to the general population (18). In one report 91% of laboratory exposures to *Brucella* were related to manipulation of isolates outside a BSC; however, the remaining 9% of exposures involved laboratory workers who manipulated cultures exclusively within a BSC. These exposures may be the result of poor airflow, disturbance of laminar flow, or other unrecognized mechanical or work practice failure. Therefore, all testing of suspected BT agents should be conducted in a BSL-3 laboratory or within a BSC using BSL-3 precautions until appropriate rule-out testing has been conducted (2).

Test algorithms have been developed for the rule out of suspected BT agents. Fortunately, these include a limited number of phenotypic and biochemical tests that can be conducted manually within a BSC. Specific guidelines for the rule out each BT agent can be found on the ASM website for sentinel level clinical laboratory protocols for suspected biological threat agents and emerging infectious diseases (https://www.asm.org/index.php/guidelines/sentinel-guidelines). Any procedures that have the potential to generate aerosols or droplets should be avoided. This includes the use of vortex, centrifugation and, forceful pipetting to make bacterial suspensions. If these procedures are necessary, it is preferred that they be carried out within a BSL-3 laboratory with negative air pressure. At a minimum, sealed rotors and tubes should be

used for required centrifugation steps, and these vessels should not be opened outside of a BSC. Importantly, isolates or cultures of suspected BT agents should not be identified using automated identification systems (e.g., VITEK, Phoenix, etc.) because of the increased risk of laboratory acquired infections associated with use of these systems (see section 4.8.3, "**Special Considerations – The Use of Microbial Identification Systems for High-Risk Pathogen Identification** "). Viral cultures should not be set up on specimens if Category A viral agents (e.g. hemorrhagic fever viruses, variola, etc.) are suspected. If these agents are detected in a clinical specimen using a direct detection technology such as PCR, the results are considered presumptive. Virial culture should not be attempted and the specimens must be referred to an LRN reference laboratory for confirmation.

4.8.3 Special Considerations – The Use of Microbial Identification Systems for High-Risk Pathogen Identification

Clinical laboratories commonly utilize automated systems for the routine identification of bacteria, viruses, and fungi. These systems rely on biochemical and phenotypic properties, molecular, or protein-based analyses to identify these microorganisms. The level of automation and specimen type required (e.g. primary clinical specimen versus cultured pure isolate) impacts the level of risk associated with each test system. Additionally, each test system or methodology has specific limitations related to the identification of BT agents which can result in the misidentification or outright failure to identify these organisms. Indeed, misidentification of a BT agent is identified as an independent risk factor for laboratory acquired infections with these agents (16). It is important to have a thorough understanding of these risks and limitations when developing a laboratory protocol for the identification of potential BT agents, and when developing a response to the unexpected identification of these microorganisms.

4.8.3.1 Automated Phenotypic Identification Systems

Automated phenotype-based identification systems (e.g., VITEK-2, bioMerieux; Phoenix Automated Microbiology System, BD; MicroScan, Beckman Coulter) require a pure bacterial or fungal isolate for analysis. In many cases, this necessitates subculture of a single colony of interest to achieve sufficient biomass for testing and to ensure purity of the isolate. This step not only exposes the technologist to potentially infectious agents through manual subculture, but also creates a high concentration culture of the isolate which further increases the risk of infection. Manipulation of *Brucella* spp., including manual subculture carries a high risk of exposure and has been associated with frequent laboratory-acquired infections (16). Therefore, manipulation of any suspected BT agent should be carried out exclusively within a BSC until definitive rule-out testing has been completed. Preparation of the test inoculum may be manual or automated (e.g. Phoenix AP, BD) but typically involves aerosol-generating procedures such as vortexing, mixing, or pipetting to prepare a high concertation suspension of the isolate. Careful preparation of the test inoculum in a BSC can reduce the risk of aerosol transmission; however, some test systems also include on-board liquid handling for panel inoculation and may generate aerosols during automated analysis. The combination of concentrated bacterial suspensions and the potential for generating aerosols results in a high risk of laboratory acquired infection when using these test systems.

In cases where potential BT agents are not immediately recognized, automated biochemical identification systems have yielded unreliable results. Many of the BT agents are fastidious, slow-growing, or biochemically inert and do not generate an adequate or reproducible biochemical profile. In addition, BT agents are often absent from the organism database of commercially available test systems and therefore cannot be identified (16, 28). Combined, these factors can result in low-confidence identification scores or misidentification as other, more common organisms in the systems' database. Recent examples of this include clinical isolates of *Y. pestis* identified as *Acinetobacter lwoffii*, *Pseudomonas luteola*, and *Yersinia pseudotuberculosis* by three different commercially available systems (29). Similarly, isolates of *B. mallei* and *B. pseudomallei* are frequently misidentified as *Burkholderia cepacia* or numerous other nonfermenting organisms including *Acinetobacter*, *Pseudomonas,* and *Moraxella* species by automated identification systems (28, 30, 31). Finally, isolates of *Brucella melitensis* and *Brucella suis* have been misidentified as *Ochrobactrum anthropi* or *Bergeyella zoohelcum* (32-34). These misidentifications can be misleading to the clinician and put laboratory workers at risk of exposure and infection (16, 32).

Given the risk of infectious aerosol and unreliable results, the use of automated identification systems should be avoided if a BT agent is suspected. If an isolate is unexpectedly identified as a BT agent by an automated system, manual testing in accordance with *ASM Sentinel Level Clinical Laboratory Guidelines* should be conducted to rule out a BT agent. If a BT agent cannot be ruled out using the guidelines, the isolate should be referred to the appropriate LRN reference laboratory for confirmation. A risk assessment should be conducted to determine the extent of potential exposure within the lab, including identification of laboratory workers at highest risk for exposure who would be candidates for serologic screening and/or post-exposure prophylaxis if a BT agent is confirmed.

4.8.3.2 MALDI-TOF MS Identification Systems

Over the past 10 years, matrix assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry has been increasingly adopted as a first line identification system for bacterial isolates. Characteristics including rapid, accurate identification of bacteria and yeast, as well as a low cost per identification have driven initial uptake in large academic or reference laboratories capable of evaluating and validating this new technology. The recent FDA-clearance of two MALDI-TOF systems, the Bruker Biotyper and VITEK MS, has made this technology more widely available to medium and small-size hospital laboratories.

Similar to automated biochemical identification systems, MALDI-TOF requires a pure bacterial or fungal isolate for analysis. However, unlike these systems, a single well-isolated colony is sufficient for identification. This eliminates the need for subculture and preparation of high concentration suspensions, thereby reducing the risk of laboratory acquired infection during pre-analytic steps. The actual identification of isolates requires ionization of the isolate, which can generate potentially infectious aerosols within the MALDI-TOF instrument. These aerosols could be released into the lab through external venting of the MALDI-TOF vacuum tube if the HEPA filter is damaged, or when retrieving the target plate from the instrument following analysis. Therefore, it is critical that any potentially infectious organism has been inactivated (i.e. is non-viable) or removed prior to analyzing with MALDI-TOF.

Several methods have been evaluated to inactivate vegetative bacterial cells as well as highly resistant endospores such as those produced by *B. anthracis*. A standardized "tube

extraction method" (suspension of an isolate in 70% ethanol, followed by centrifugation and resuspension of the bacterial pellet in a 1:1 mixture of 100% acetonitrile and 70% formic acid) has been found to effectively inactivate vegetative cells and generate high quality mass spectra (35). Unfortunately, this method is laborious for routine analysis of isolates and does not effectively inactivate endospores of *B. anthracis* (36). Pre-treatment of isolates with various concentrations of trifluoroacetic acid (TFA) have demonstrated more effective inactivation of endospores, but poorer quality mass spectra when compared to ethanol inactivation (37, 38). On-label use of the FDA-cleared MALDI-TOF systems calls for direct application of α-cyano-4-hydroxycinnamic acid (HCCA) matrix dissolved in an aqueous mixture of acetonitrile (ACN) and TFA to a bacterial isolates for analysis. This matrix solution alone is sufficient to inactivate a number of vegetative bacteria including BT agents (e.g. *B. melitensis*, *F. tularensis*) and some endospore-forming *Bacillus* spp., excluding *B. anthracis* (39). An additional step involving the addition of 70% formic acid (FA) to the bacterial isolate prior to HCCA matrix also fully inactivated both the vegetative cells and *B. cereus* endospores tested in this study.

These methods are convenient for laboratory workflow and provide a reasonable level of safety during routine preparation of isolates for MALDI-TOF analysis. This is important since BT agents may often be unexpected until they are identified. However, risks associated with these standard approaches include incomplete mixing of bacteria with matrix, bacterial cells outside the MALDI target plate zone that may not come in contact with matrix solution, or a high endospore burden that is not fully inactivated by FA or matrix. Any one of these factors could result in residual viable organism and pose a risk of exposure. Indeed, incomplete inactivation of *B. anthracis* endospores using the tube extraction method (described above) has been reported, and in one case resulted in the inadvertent release of potentially viable *B. anthracis* from a BSL-3 laboratory (35, 36). Therefore, when a BT agent is suspected, added precautions are recommended. These include i) pre-treatment of the isolate using the "tube extraction method" and ii) filtration of the resulting extract through a 0.1- 0.2 µm filter to remove any remaining viable cells or endospores. This process inactivates and lyses vegetative cells, thereby liberating the intracellular proteins that are the primary substrate for MALDI TOF identification. These proteins will pass freely through the filtration step while any remaining viable bacteria or endospores will be retained (35, 36, 40). As with any suspected BT agent, all pre-treatment preparation steps should be carried out in a BSC and with the use of a sealed centrifuge rotor to prevent aerosol in the event of tube breakage or leak.

Organism identification via MALDI-TOF is dependent on two key components. First, the MALDI-TOF must generate a high quality spectral profile from the organism being analyzed. This can be impacted by the solvents used (e.g. TFA vs. ethanol, discussed earlier) or by physical properties of the organism itself such as integrity of the cell wall or the presence of endospores. Organisms such as yeasts, mycobacteria, or endospore may benefit from additional pre-processing to obtain high-quality spectra (41-43). Once a quality spectrum is obtained, it is compared to a reference database of spectra generated from characterized clinical isolates and type strains. If a sufficient match is not found, the result will be either a failure to generate an organism ID or a low confidence ID. Alternatively, a closely related species may be incorrectly reported if the isolate is not present in the MALDI-TOF reference database. Current FDA-cleared IVD databases for both the Bruker Biotyper and Vitek MS lack reference spectra for BT agents. This can lead to missed or misidentification. In one instance, the incorrect identification of a urinary isolate as *Burkholderia thailandensis* resulted in laboratory exposure to the BT agent *B. pseudomallei* (27). Conversely, in another report an isolate of *Yersinia pseudotuberculosis*

was misidentified as the BT agent *Y. pestis* using MALDI-TOF (44). Due to these limitations, it is of paramount importance to remain vigilant for potential BT agents and perform the standard biochemical rule out testing algorithm when there is suspicion regardless of the identification reported by currently FDA-cleared MALDI-TOF databases.

Research use only (RUO) databases are available specific groups of organisms, including BT agents, and are better suited to identifying these organisms. However, even these BT-agent-specific databases may fail to differentiate between closely related species (40). Importantly, the use of these databases requires extensive validation studies which are impractical for most laboratories given the additional biosecurity and biosafety requirements for culture and storage of these agents.

4.8.3.3 Molecular Identification Methods

Molecular methods including nucleic acid amplification tests (NAATs) enable identification of BT agents in primary clinical specimens. This eliminates many of the risks associated with routine culture (e.g. multiple manual manipulations, cultivation of pure cultures with a high concentration of organism) and can be especially useful for the detection of fastidious or non-cultivable bacterial or viral pathogens. Further, the use of NAATs provides both higher sensitivity and significantly more rapid turnaround time than bacterial or viral culture methods.

Extraction and purification of nucleic acids from clinical specimens is a key factor in success of downstream amplification and detection steps. Manual extraction using columns is routinely conducted using centrifugation, and should be carried out in a sealed rotor. While effective, manual extraction methods are time consuming and require several manual steps which increase the chance of laboratory exposure. High-throughput automated extraction platforms (e.g. NucliSens easyMAG (bioMérieux), MagNA Pure (Roche)) require minimal hands on steps, thereby reducing the risk of direct exposure, and are utilized by many modern laboratories. Unfortunately, these systems have demonstrated variable performance in extracting nucleic acids from inactivated BT agents, including *B. anthracis* endospores, in buffer or blood matrix (45). Further, automated liquid handling steps including the addition lysis buffer and other reagents, as well as sample mixing steps have the potential to generate infections aerosols.

Two lysis buffers commonly used in automated and manual extraction methods effectively inactivated a number of viral pathogens, including Marburg, Ebola, Rift Valley fever, and Venezuelan equine encephalitis viruses (46). Similarly, automated and manual extraction platforms reliably inactivated *Brucella* spp. at the highest concentrations tested (10^7 CFU/mL) (47). In contrast, inactivation of *B. anthracis* endospores was variable and incomplete, ranging from as little as 1 $\log_{10}$ to as high as 5 $\log_{10}$ reduction in viable spores (48). These data suggest that nucleic acid extraction methods are capable of inactivating labile enveloped viruses and vegetative bacterial cells, thereby providing a reasonable level of safety when working with routine clinical specimens that may contain these pathogens. However, high concentration suspensions containing presumed BT agents and specimens or cultures containing endospores should not be subjected to extraction.

There are currently no FDA-cleared NAATs that specifically detect BT agents. Few clinical laboratories have developed LDTs for these agents because of the relatively rare occurrence and difficulty in conducting validation studies with these highly infectious organisms. A multiplex panel capable of identifying 16 highly infectious or BT agents in ~1h has been

developed (FilmArray BT Panel). The BT Panel is a sample-to-answer test that includes a sealed, single use consumable containing all reagents for nucleic acid extraction, amplification, and detection of the target. This approach provides the greatest level of safety when working with specimens that may contain BT agents. The BT Panel was granted Emergency Use Authorization (EUA) in October of 2014 during the Ebola outbreak in Western Africa to aid clinical laboratories in rapid assessment of clinical specimens from symptomatic patients with recent travel to an endemic area or exposure to an infected individual. Limited evaluations demonstrated a sensitivity of 85-91% for detection of Ebola virus in blood and urine specimens (49, 50). Other targets on the panel have not been thoroughly evaluated with clinical specimens.

Validation and ongoing proficiency testing for use of the BT Panel or similar research use only (RUO) tests is a significant challenge for most clinical laboratories. Consideration must also be given to maintenance of equipment and training for proper use of PPE and handling of highly infectious specimens. LRN reference laboratories, in conjunction with CDC, have validated molecular tests specific for many of the viral and bacterial agents considered to be highly infectious or biological terrorism threats. Suspicion of a BT agent should be communicated to the laboratory by the ordering clinician so proper precautions can be taken to ensure safety of laboratory workers. Regardless of whether or not a BT agent is detected using LDT or EUA assays, the specimen should be referred to a local LRN laboratory for definitive identification.

4.8.3.4 Total Laboratory Automation

An exciting advancement in microbiology is the introduction of automated specimen processing and total laboratory automation (TLA). Two systems, the BD Kiestra and Copan WASP/WASPLab offer semi- and total-lab automation solutions for microbiology. Both systems have the potential to provide enhanced safety through reduced contact with primary specimens and cultured isolates; however, each system also has specific shortcomings that must be considered. Importantly, specimens known or presumed to contain BT or other highly infectious agents should not be processed using automated systems. These specimens should be taken off-line and manually processed using pathogen-specific guidelines which may include the use of a BSL-3 suite, if available, or work exclusively within a class II BSC using BSL-3 precautions.

A major advantage of TLA is the automated front-end processing of primary specimens. For some specimen types (e.g. urine, sputum, stool), no direct technologist interaction is necessary. The WASP is a 90% enclosed system capable of all primary processing steps including labeling of plates, vortexing or centrifuging of the specimen, uncapping and recapping, and inoculation of liquid or solid media via reusable steel inoculating loop. A HEPA filter vacuum is located near the tube uncapping and inoculation components to capture infectious aerosols generated during specimen plating. The BD Kiestra automated inoculation module (InoqulA) is capable of uncapping, recapping, and pipette-based inoculation of plating media. Inoculated plates are closed prior to bead-based streaking to reduce aerosols, and the entire InoqulA module is enclosed with air vented through a HEPA filtration system. Specimens not amenable to automated plating can be processed in an integrated BSC prior to automated streaking (51).

Beyond front-end processing, TLA conveyors and incubators further reduce physical contact with potentially infectious agents and virtually eliminate the risk of dropping trays of

inoculated plates during manual transit of cultures throughout the laboratory (a truly cataclysmic event). WASPLab and BD Kiestra TLA both incorporate high resolution imaging systems that enable "telemicrobiology", a process whereby images of culture plates can be viewed on computer screens. This eliminates the risk of exposure from opening and examining plates on the benchtop during routine work-up. Image analysis can be used to preliminarily identify colonies with phenotypic characteristics consistent with BT agents such as slow growth rate, failure to grow on blood agar, or a dry, wrinkled appearance. These cultures can be flagged and managed according to BT-agent-specific protocols. Future developments in automation may well eliminate all manual interaction with cultures as automated colony picking, subculture, and preparation of colonies for MALDI-TOF analysis become available.

While these safeguards have been engineered to reduce risk of laboratory exposure, there have been no definitive studies comparing the safety of WASP or BD Kiestra systems to routine laboratory practices including standard precautions in conjunction with the use of BSCs for primary processing and culture work-up.

Despite the advantages of TLA systems, fully automated specimen processing is still not applicable to the majority of clinical specimens received in the microbiology laboratory. Tissues, positive blood culture broths, solid specimens, and specimens collected with standard wound fiber swabs are not amenable to current automated inoculation systems and account for up to 50% of specimens received by the laboratory (52). Therefore, the laboratory technologist will continue to be integral in primary processing and work-up of cultures. Mechanized processing can also be subject to failure. Use of different sized agar plates not recognized by the plate-handling robots can result in crushing or breaking. Failure to adequately recap specimen tubes (e.g. cross-threading) prior to vortexing can result in significant spillage and generation of aerosols. While these spills are contained within the instrument, thorough decontamination is difficult given all the mechanized instrumentation and surfaces. Laboratories should consult manufacturers for recommended disinfectants that will not damage the various instrumentation components and for recommended routine decontamination practices. Ultimately, each laboratory must develop protocols to routinely monitor for contamination of surfaces within the instrument and a standardized method for both routine and post spill decontamination. A simple approach to environmental monitoring is to process a group of 8-12 uninoculated nutrient broths using the laboratory automation protocol for clinical specimens. This process should encompass all automated processing steps including decapping of the media tube, sampling of the specimen with onboard loops or pipette tips, and inoculation plating media. The inoculated plating media, as well as the nutrient broth tubes should be incubated for 48-72 h and examined for bacterial or fungal growth. If growth is observed, this would indicate contamination of one or more components of the automation. Environmental sampling of each specific component may be appropriate if a specific point source of the contamination is sought; however, full decontamination of the system should be conducted and the system should be retested for sterility prior to reinitiating clinical testing. If there is a recognized spill, appropriate time should be permitted for aerosols to settle prior to opening the automated specimen processing enclosure or cabinet. This time is typically 20-30 minutes, but is also impacted by the air exchange rate specified by the manufacturer.

5. BIOSECURITY

Biosecurity in the context of microbiological and biomedical science laboratories refers to security measures taken by such facilities to prevent the theft, intentional or unintentional release, unauthorized access to, or loss of infectious agents. Examples of biosecurity measures employed in sentinel level laboratories are summarized below, along with a brief overview of regulations pertaining to the transportation, maintenance, and destruction of select agents.

5.1 General Requirements for Sentinel Level Laboratories

Because of the potential risks to public health posed by the infectious agents handled in sentinel level laboratories, these facilities must adopt stringent biosecurity measures to ensure that clinical specimens, cultures and stocks of infectious agents, and infectious biomedical wastes cannot be lost, stolen, or otherwise tampered with, either accidently or intentionally. To curb unauthorized access to the laboratory area, most sentinel level laboratories are equipped with access control devices such as key card or key fob scanners. Under normal operating parameters, the doors of the laboratory are kept locked until an authorized person scans his or her card/fob, at which point the doors will be unlocked, allowing for access. In addition, surveillance cameras are sometimes placed at entry points to the laboratory as well as sites used to store pathogen stocks and infectious wastes to monitor access to these places.

Visitors to laboratories should be required to sign logs, which can be referenced if an incident occurs that may involve a visitor to the laboratory. Locks, either electronic or pad-locks, should be used on refrigerators, freezers, or other devices used for the temporary storage of suspected BT agents; however, some laboratory directors may choose to lock freezers and other containers used to store routine isolates of human pathogens. In addition to the physical means used to prevent unauthorized access to pathogens, facilities should also adopt emergency management plans aimed at mitigating the consequences of intentional or accidental agent release and, included among this information, is a clearly defined process for alerting public health professionals and law enforcement agencies. Regardless of the nature of the agents handled, a thorough risk assessment should be used to guide implementation of a sentinel level laboratory biosecurity plan.

5.2 Transportation of BT Agents

Cultures of select agents and other high-consequence pathogens must be transported in accordance with applicable regulations defined by the U.S. Department of Transportation, the International Air Transport Association, and other regulatory bodies. These agents must be packaged and shipped as Category A Infectious Substances unless otherwise stated. An exception to this rule is for avirulent or virulence-attenuated strains of some agents (e.g., vaccine strains). Additional information regarding the transport of BT agents can be found on the Federal Select Agent website under the heading "General questions about transport of select agents and toxins" found here: https://www.selectagents.gov/faq-transfers.html.

5.3 Maintenance and Destruction of Select Agents

Possession and transfer of BT agents is governed by Federal Select Agent Program regulations contained in the Code of Federal Regulations (7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73) and is enforced by the Federal Bureau of Investigation (FBI). The full regulations for handling, reporting, and transfer of select agents can be found at https://www.selectagents.gov/index.html. Among these regulations, 42 CFR Part 73 pertains to select agents and toxins of concern to public health and are most applicable to clinical laboratories dealing with human specimens.

Only laboratories certified by the department of Health and Human Services (HHS) may legally possess select agents, including those classified as Tier 1 BT agents. This certification includes a risk assessment of the laboratory and personnel with an emphasis on biosafety and biosecurity. Specific examples of these measures include the requirement for a federal background check for any individuals that will have access to the select agents and controlled access (e.g., card access, PIN code, etc.) to the general laboratory as well as individual freezers where agents are stored. Further, protocols must be in place to track all cultures and freezer stocks of select agents. This is commonly achieved using daily inventory and log sheets indicating the number and location of cultures and strains present in the laboratory. Because of these rigorous regulations, certification is beyond the scope of clinical laboratories and is reserved for select academic and national research centers.

If a laboratory presumptively identifies a select agent, or a BT agent cannot be ruled out, the appropriate public health and LRN reference laboratory should be notified immediately. The clinical specimen or isolate should be referred to the LRN reference laboratory for confirmatory testing in accordance with appropriate packaging and shipping guidelines (see "*Shipping*" below). A laboratory may keep clinical specimens or isolates until a BT agent has been definitively identified; however, depending on the level of suspicion further testing or manipulation of the clinical specimen may be limited to tests essential for patient management. Once a definitive identification is made, the clinical specimen and any associated cultures must be destroyed or transferred within 7 days to a laboratory certified by HHS to maintain select agents. Definitive identification of Tier 1 agents must be reported to the Federal Select Agent Program within 24 h. Additionally, the laboratory director or supervisor must fill out Animal and Plant Health Inspection Service (APHIS)/CDC Form 4A within 7 days of identification of a select agent or toxin and return this form to the CDC. A list of specific select agents, forms and contact information can be found at https://www.selectagents.gov/form4.html. If the agent will be transferred to a HHS certified laboratory, APHIS/CDC Form 2 must be completed and approved by the CDC.

6. BIOMEDICAL WASTE MANAGEMENT

Biomedical waste, also called biohazardous waste, infectious waste, medical waste, and regulated medical waste, is biologically contaminated waste that comprises an array of subcategories, including liquid wastes, pathological wastes, sharp wastes, non-pathological and non-sharp solid wastes, and chemically and radioactively contaminated biological wastes, among others. Descriptions of these wastes and methods used to decontaminate them are described in the sections below. It is important to note that all applicable institutional, local, state, and federal

guidelines for biomedical waste disposal must be followed for the disposal of these substances, as biomedical waste requirements differ from institution to institution and from region to region.

6.1 Descriptions of Biomedical Wastes

6.1.1 Liquid Wastes

Liquid biomedical wastes can include a variety of substances, including:
- Human blood and blood products;
- Human body fluids other than blood (e.g., cerebrospinal fluid, peritoneal fluid, etc.);
- Some types of non-human animal body fluids (e.g., blood and other fluids from animals experimentally or naturally infected with infectious agents);
- Liquid cultures of microorganisms (e.g., broth cultures of bacteria and fungi);
- Spent cell culture media used for propagation of human and non-human primate cell lines assigned to RG-2 and above;
- Spent cell culture media used in the propagation of viruses and other obligate intracellular pathogens;
- Unused live-attenuated vaccines; and
- Other biologically contaminated liquid substances deemed to be biohazardous by the laboratory director and/or other regulatory oversight / governing body.

6.1.2 Pathological Wastes

Pathological wastes are defined as biomedical wastes that include human tissues such as amputated appendages, organs and organ fragments, biopsies, bone, and other body parts removed during surgery or autopsy. Animal tissues, including those mentioned above, may also be considered pathological waste if they are known to, or are suspected of, containing infectious agents.

6.1.3 Sharp Wastes

Sharp wastes, or "sharps," are biomedical waste items that are capable of puncturing a plastic disposal bag and potentially resulting in a puncture injury to the individual handling the waste items. A variety of sharp wastes are generated as a result specimen processing, culture inoculation, culture work-up, and other microbiological manipulations. These wastes include, but are not limited to:

- Syringes;
- Needles;
- Blood collection devices (e.g., winged venipuncture sets);
- Blood transfer devices (e.g., devices used to transfer blood from a syringe to a blood culture bottle);
- Scalpel blades, including single-piece, one-time use scalpels;
- Microscope slides and coverslips;
- Pasteur pipets;

- Micropipet tips;
- Wooden applicator sticks;
- Rigid disposable inoculating loops and needles;
- Disposable serological and volumetric pipets;
- Biologically contaminated broken glass or shattered rigid plastic; and
- Other items that may tear or puncture a plastic disposal bag or human skin.

6.1.4 Non-Pathological and Non-Sharp Solid Wastes

Non-pathological and non-sharp solid wastes, sometimes referred to as "soft" wastes, include a number of solid wastes that, under normal waste handling conditions, will not puncture a plastic disposal bag or human skin. These wastes include, but are not limited to:

- Plastic culture dishes (e.g., agar plates used for bacterial and fungal propagation);
- Plastic culture flasks (e.g., cell culture flasks);
- Plastic culture plates (e.g., 6-well cell culture plates);
- Rounded-corner multi-well plates (e.g., 96-well broth microdilution panels);
- Contaminated gloves;
- Contaminated disposable PPE (e.g., disposable laboratory coats);
- Contaminated absorbent pads;
- Contaminated paper towels; and
- Other items that will not tear or puncture a plastic disposal bag or human skin under normal waste handling conditions.

6.1.5 Chemically and Radioactively Contaminated Biomedical Wastes

Biomedical waste disposal companies, incinerators, and landfills have strict guidelines with regard to the permissible amounts of certain chemical substances and detectable radiation that may be disposed of in combination with biomedical wastes. Most microbiological wastes generated in medical and public health laboratories are not contaminated with large quantities of hazardous chemicals and/or radioactive substances, but the potential does exist.

6.2 Biomedical Waste Decontamination and Disposal

6.2.1 Disposal of Liquid Wastes

Disposal of liquid biomedical wastes is usually accomplished by inactivation of the biological agents contained within them followed by disposal of the decontaminated fluid down a dedicated drain such as a "dirty sink" or latrine. A number of chemical and physical agents can be employed for liquid decontamination, including sodium hypochlorite (bleach), quaternary ammonium compounds (e.g., Micro-Chem Plus^{TM}), and autoclaving. When chemical disinfectants are used, it is prudent that manufacturer-specified disinfectant concentrations, contact times, and other parameters are followed to ensure disinfection of biological hazards present within the liquid. In addition, the nature of the infectious agents present within the liquid waste and the composition of the liquid waste itself can affect the performance of a disinfectant

solution, as not all disinfectants are universally microbicidal and some disinfectants are partially or completely inactivated by certain types of organic materials (e.g., blood, feces, etc.). In all instances, the combined volume of liquid waste, concentrated disinfectant, and diluent, if used, must not exceed the manufacturer-recommended concentration of disinfectant in the total volume of liquid waste. In other words, if the manufacturer states that a final concentration of 5% (vol/vol) is required to inactivate one or more infectious agents within a liquid, the total volume of liquid waste contained within a disposal vessel must not dilute the disinfectant below 5%.

To avoid dilution of the disinfectant beyond the effective concentration, laboratories may choose to fill liquid collection containers with concentrated disinfectant solutions and add liquid wastes until a total volume that yields a still-effective concentration of the disinfectant is reached. If only a small volume of liquid waste is to be generated, a suitable diluent (e.g., water) may be added to the disinfectant concentrate prior to disposal of the liquid biomedical waste. Again, the total volume of liquid waste must not dilute the disinfectant beyond its effective concentration. Once filled, liquid waste vessels must be allowed to remain undisturbed for a set amount of time (e.g., 30 minutes) to ensure inactivation of infectious agents prior to drain-disposal. Another means of chemical disinfectant-based inactivation of liquid biomedical wastes utilizes absorbent sachets or powders that simultaneously gel and disinfect liquids. Following the manufacturer-recommended contact time, absorbed or gelled liquids can be disposed of in the solid biomedical waste stream. Again, as mentioned previously, disinfectants (liquid, solid, etc.) should be used in accordance with the manufacturer's directions for use and prior to the expiration date printed on the disinfectant's original container (see section 4.6.1**, "Levels of Disinfection and Types of Chemical Disinfectants"**.

The most commonly used physical decontamination method for inactivation of liquid biomedical wastes is autoclaving. In general, liquid wastes must be collected in autoclavable plastic or glass containers that are fitted with closures (e.g., foil caps) prior to steam sterilization. Strips of autoclave tape should be used to secure closures onto containers, and tightened screw-caps should not be used so as to avoid potentially destructive pressure buildup within the vessel during the autoclave cycle. To avoid waste "boil-over" during depressurization of the autoclave chamber, it is important that collection vessels are not filled to maximum capacity. As a rule of thumb, liquid waste containers should only be filled half way. Other considerations for autoclaving liquid wastes include the autoclave cycle type, the time needed to effect waste sterilization, the use of an autoclavable secondary container (e.g., autoclave pan or bin), autoclave monitoring, and operator safety. In addition, liquid wastes containing bleach and certain other disinfectants should not be autoclaved, as they can release vapors that could damage the autoclave or prove to be harmful to laboratory personnel. To determine if a disinfectant is compatibile with autoclaving, refer to the product's documentation or contact the manufacturer.

Most modern autoclaves are able to be programmed to accommodate several autoclave cycle types that are to be used for sterilization of different items, including liquids and solids. When autoclaving liquids, a cycle that slowly depressurizes the chamber following a run must be used to avoid liquid boil-over or eruption. Many autoclave manufacturers recommend autoclaving liquid biomedical wastes for at least 1 hour prior to disposal of the waste down a sanitary sewer drain. The choice to autoclave liquid wastes for less than manufacturer-recommended times should be backed up with evidence that shorter cycles effectively decontaminate infectious agents that are routinely disposed of in this fashion. The use of an

autoclavable secondary container such as an autoclave bin in which the primary liquid waste collection vessel can be placed is highly recommended. Such containers are usually designed for easy carrying and handling of items to be autoclaved and afford containment of liquids that may spill from the primary disposal vessel. Regular (e.g., weekly) monitoring of autoclave performance is essential to ensure proper autoclave function. Biological indicators, such as vials containing *Geobacillus stearothermophilus* spores, are used to assess autoclave function and sterilization parameters; the use of autoclave indicator tape alone is insufficient for this purpose. Logs of autoclave performance parameters must be kept and regularly reviewed by laboratory supervisors or other authorized personnel to ensure that autoclaves are functioning properly. In addition, shifts or trends in autoclave performance can be warning signs that maintenance of the autoclave is needed. Finally, autoclave operators must be thoroughly trained and their competency must be periodically assessed. Appropriate PPE, including a face shield, thermally protective gloves or mittens, and a thermally protective apron should be worn by personnel when manipulating autoclaved liquids, as materials exiting the autoclave are very hot and can cause severe burns.

Some liquid wastes, such as blood culture bottles and tube-cultures of bacteria and fungi sealed with screw caps, can be directly discarded into containers (e.g., solid-walled boxes or buckets) designed for disposal of these wastes. If, however, RG-3 or RG-4 agents are contained within the liquid waste, bottles and tubes must be autoclaved or otherwise decontaminated prior to final disposal. Please consult public health professionals, biological safety officers, and/or other regulatory specialists for more information.

6.2.2 Disposal of Pathological Wastes

Pathological wastes are not usually terminally disposed of by medical or public health laboratories, but instead are disposed of by licensed medical waste contractors. Tissue wastes should be containerized according to waste contractor, institution, and local/state/federal guidelines prior to pick up. Usually, pathological waste disposal entails decanting chemical fixatives or bulk fluids prior to placement of the tissue within one or more bags, boxes, or buckets. The most common method of terminal disposal of pathological wastes is incineration, but other methods, including alkaline hydrolysis (i.e., tissue digestion), may also be used. In some instances, including situations in which tissues are known to contain high-risk biological agents (e.g., RG-4 viruses), pathological wastes may be required to undergo autoclaving prior to terminal disposal.

6.2.3 Disposal of Sharp Wastes

Discarded sharps should be contained within puncture-resistant containers designed for the purpose of sharps disposal. Numerous styles and sizes of sharps containers are available from a variety of suppliers, including medical waste disposal contractors. Prior to use, sharps containers should be fitted with lids and closely inspected to ensure that the containers and their lids are structurally sound. Sharps containers must be placed as close to the point of sharp waste generation as possible and must not be perched on unstable surfaces or suspended in unapproved holders, as misplacement or misuse may result in spillage and sharps-associated injuries. In addition, sharps containers must never be overfilled; instead, sharps containers must only be filled to the "full" line drawn on the container label. Forcing sharps into full containers can result

in sharps-associated injuries that can potentially lead to laboratory-acquired infections. Finally, needles should never be re-capped and any built-in sharps safety devices (e.g., needle sheaths) must be engaged prior to disposal. Depending upon the nature of the biological substances contaminating the sharp waste items, decontamination (e.g., by autoclaving) of filled sharps containers may be necessary prior to disposal in the institution's biomedical waste stream.

6.2.4 Disposal of Non-Pathological and Non-Sharp Solid Wastes

"Soft" wastes are usually contained within plastic biohazard bags, bag-box units, or other containers designed to accommodate these waste types. To avoid rupture and spillage of waste bags, double-bag microbiological wastes and fill bags to two-thirds of their maximum capacity. Some waste items, including cultures of RG-3 and RG-4 agents and specimens containing these pathogens, should be autoclaved prior to terminal disposal if an autoclave is available on site. Autoclavable biohazard bags made of high-density polyethylene or similar polymers should be used for this purpose; standard biohazard bags should not be used for autoclaving wastes, as they will melt within the autoclave chamber and the waste contained within them will spill. Most laboratory supply vendors sell autoclavable waste bags that are available in a variety of sizes and colors (e.g., red, orange, and colorless). Prior to autoclaving, bags must be loosely closed to permit steam penetration into the bags to effect sterilization of waste contents. To avoid manipulation of bags containing contaminated wastes, some laboratories utilize autoclavable bag holders that, along with the bag lining them, can be autoclaved. Following sterilization, bags should be sealed and discarded according to institutional and governmental guidelines. As with autoclaving liquid and other biomedical waste types, autoclave parameters must be monitored for sterilization of solid wastes. In general, bags of waste should be autoclaved using standard parameters (autoclave temperature and pressure equal to 121°C and 15 p.s.i., respectively) for at least 1 hour; however, large waste loads should be autoclaved for longer periods of time. For laboratories that do not have an autoclave on site, medical waste can be decontaminated at a contracted medical waste treatment facility. Medical waste must be placed into appropriate medical waste shipping containers and packaged according to applicable regulatory standards (53). A risk assessment should always be conducted to determine whether waste should be decontaminated off-site or on-site.

6.2.5 Disposal of Chemically and Radioactively Contaminated Wastes

The means by which chemically and radioactively contaminated biomedical wastes should be disposed of depends upon the types and quantities of chemical(s) and/or radioactive substances present within the waste. For some chemicals (e.g., formalin), small volumes may be permitted to be disposed of along with the biomedical waste without prior treatment of the waste to render them chemically inert. However, large volumes of such chemicals must never be disposed of as biomedical waste. Instead, the biomedical waste contractor, the institutional biological and chemical safety officers, and/or other regulatory specialists / agencies must be consulted to provide guidance for mixed waste disposal. For biomedical wastes contaminated with radioactive isotopes, most medical waste disposal contractors will not accept wastes that contain detectable levels of radiation. Instead, it is the responsibility of the waste generator to ensure that radioactive biomedical wastes have been allowed to decay below the limit of detection prior to disposal as biomedical waste. As with chemically contaminated biomedical

wastes, please consult institutional and governmental radioactive waste disposal specialists for advice pertaining to disposal of radioactive biomedical waste disposal.

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Appendix

Association of Public Health Laboratories Biosafety-Related Checklists

1. APHL Biosafety Resources Page:
   https://www.aphl.org/programs/preparedness/Biosafety-and-Biosecurity/Pages/BB-Resources.aspx

2. APHL Biorisk Management Checklist:
   https://www.aphl.org/programs/preparedness/Biosafety-and-Biosecurity/Documents/Clinical_Lab_Assessment_Checklist_Final.pdf

3. APHL Biosafety Culture Checklist:
   https://www.aphl.org/AboutAPHL/publications/Documents/ID_BiosafetyChecklist_42015.pdf