

2. Biological Risk Assessment and Biosafety Guidelines

2.1. Risk Assessment

The laboratory director is ultimately responsible for identifying potential hazards, assessing risks associated with those hazards, and establishing precautions and standard procedures to minimize employee exposure to those risks. Because the identity of an infectious agent is initially unknown in the clinical laboratory, the general recommendation is that the biosafety level (BSL)-2 standard and special practices in *Biosafety in Microbiological and Biomedical Laboratories*, 5th edition (1) be followed for all work in the clinical laboratory, and the Occupational Safety and Health Administration's (OSHA's) Standard Precautions (gloves, gowns, and protective eyewear) (33) and BSL-2 practices (2) be employed during handling of all blood and body fluids. Other comprehensive resources are available (34,35). Risk assessment, as outlined here and in Section 12, may determine that decreasing or increasing the BSL practices or facilities is warranted (Figure 1).

Qualitative biological risk assessment is a subjective process that involves professional judgments. Because of uncertainties or insufficient scientific data, risk assessments often are based on incomplete knowledge or information. Inherent limitations of and assumptions made in the process also exist, and the perception of acceptable risk differs for everyone. The risk is never zero, and potential for human error always exists.

Identifying potential hazards in the laboratory is the first step in performing a risk assessment. Many categories of microbiological hazards are encountered from the time a specimen is

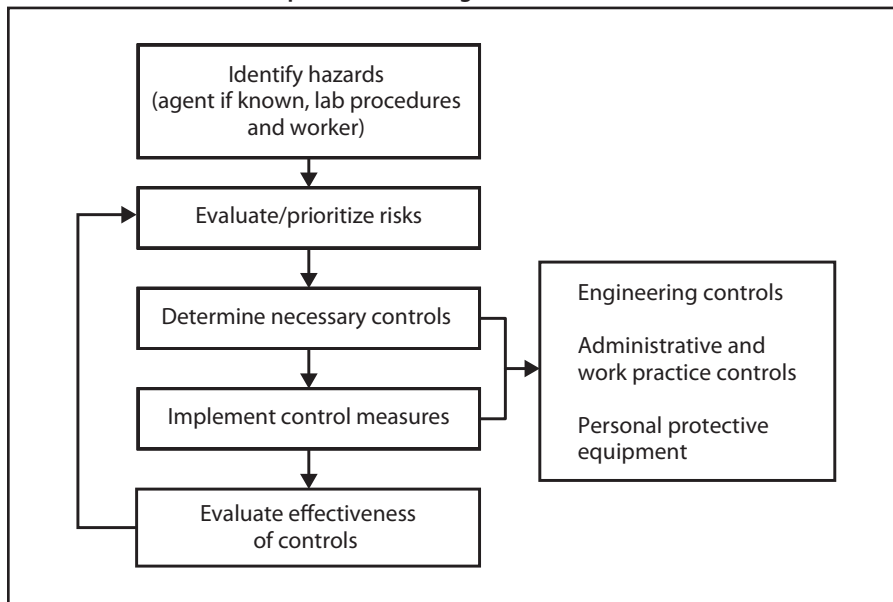
collected until it is disposed of permanently. A comprehensive approach for identifying hazards in the laboratory will include information from a variety of sources. Methods to ascertain hazard information can include benchmarking, walkabouts, interviews, detailed inspections, incident reviews, workflow and process analysis, and facility design.

No one standard approach or correct method exists for conducting a risk assessment; However, several strategies are available, such as using a risk prioritization matrix, conducting a job hazard analysis; or listing potential scenarios of problems during a procedure, task, or activity. The process involves the following five steps:

1. Identify the hazards associated with an infectious agent or material.
2. Identify the activities that might cause exposure to the agent or material.
3. Consider the competencies and experience of laboratory personnel.
4. Evaluate and prioritize risks (evaluate the likelihood that an exposure would cause a laboratory-acquired infection [LAI] and the severity of consequences if such an infection occurs).
5. Develop, implement, and evaluate controls to minimize the risk for exposure.

Standardization of the risk assessment process at an institution can greatly improve the clarity and quality of this process. Training staff in risk assessment is critical to achieving these objectives.

FIGURE 1. Risk assessment process for biologic hazards



2.1.1. Step 1. Identify the hazards associated with an infectious agent or material.

- The potential for infection, as determined by the most common routes of transmission (i.e., ingestion by contamination from surfaces/fomites to hands and mouth; percutaneous inoculation from cuts, needle sticks, nonintact skin, or bites; direct contact with mucous membranes; and inhalation of aerosols) (Table 1);
- The frequency and concentration of organisms routinely isolated, as determined by specimen type, patient data (of individual or the hospital population), epidemiologic data, and geographic origin of the specimen;
- Intrinsic factors (if agent is known)
 - Pathogenicity, virulence, and strain infectivity/communicability;

TABLE 1. Laboratory activities associated with exposure to infectious agents

Routes of exposure/transmission	Activities/practices
Ingestion/oral	<ul style="list-style-type: none"> • Pipetting by mouth • Splashing infectious material • Placing contaminated material or fingers in mouth • Eating, drinking, using lipstick or lip balm
Percutaneous inoculation/nonintact skin	<ul style="list-style-type: none"> • Manipulating needles and syringes • Handling broken glass and other sharp objects • Using scalpels to cut tissue for specimen processing • Waste disposal (containers with improperly disposed sharps)
Direct contact with mucous membranes	<ul style="list-style-type: none"> • Splashing or spilling infectious material into eye, mouth, nose • Splashing or spilling infectious material onto intact and nonintact skin • Working on contaminated surfaces • Handling contaminated equipment (i.e., instrument maintenance) • Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material • Bites and scratches from animals and insects • Waste disposal • Manipulation of contact lenses
Inhalation of aerosols	<ul style="list-style-type: none"> • Manipulating needles, syringes, and sharps • Manipulating inoculation needles, loops, and pipettes • Manipulating specimens and cultures • Spill cleanup

Source: Sewell DL. Laboratory-associated infections and biosafety. *Clin Microbiol Rev* 1995;8:389–405 (18).

- Mode of transmission (mode of laboratory transmission may differ from natural transmission);
- Infectious dose (the number of microorganisms required to initiate infection can vary greatly with the specific organism, patient, and route of exposure);
- Form (stage) of the agent (e.g., presence or absence of cell wall, spore versus vegetative, conidia versus hyphae for mycotic agents);
- Invasiveness of agent (ability to produce certain enzymes); and
- Resistance to antibiotics.
- Indicators of possible high-risk pathogens that may require continuation of work in a biological safety cabinet (BSC), such as
 - Slowly growing, tiny colonies at 24–48 hours with Gram stain showing gram-negative rods or gram-negative coccobacilli;
 - Slow growth in blood culture bottles (i.e., positive at ≥48 hours), with Gram stain showing small gram-negative rods or gram-negative coccobacilli;
 - Growth only on chocolate agar;
 - Rapid growth of flat, nonpigmented, irregular colonies with comma projections and ground-glass appearance;
 - Gram stain showing boxcar-shaped, gram-positive rods with or without spores.

2.1.2. Step 2. Identify activities that might cause exposure to the agent or material.

- The facility (e.g., BSL-2, BSL-3, open floor plan [more risk] versus separate areas or rooms for specific activities

[less risk], sufficient space versus crowded space, workflow, equipment present);

- The equipment (e.g., in the case of uncertified BSCs, cracked centrifuge tubes, improperly maintained autoclaves, overfilled sharps containers, Bunsen burners);
- Potential for generating aerosols and droplets. Aerosols can be generated from most routine laboratory procedures but often are undetectable. The following procedures have been associated with generation of infectious aerosols.
 - Manipulating needles, syringes and sharps
 - Subculturing positive blood culture bottles, making smears
 - Expelling air from tubes or bottles
 - Withdrawing needles from stoppers
 - Separating needles from syringes
 - Aspirating and transferring body fluids
 - Harvesting tissues
 - Manipulating inoculation needles, loops, and pipettes
 - Flaming loops
 - Cooling loops in culture media
 - Subculturing and streaking culture media
 - Expelling last drop from a pipette (including Eppendorff pipettes)
 - Manipulating specimens and cultures
 - Centrifugation
 - Setting up cultures, inoculating media

- Mixing, blending, grinding, shaking, sonicating, and vortexing specimens or cultures
- Pouring, splitting, or decanting liquid specimens
- Removing caps or swabs from culture containers, opening lyophilized cultures, opening cryotubes
- Spilling infectious material
- Filtering specimens under vacuum
- Preparing isolates for automated identification/susceptibility testing
- Preparing smears, performing heat fixing, staining slides
- Performing catalase test
- Performing serology, rapid antigen tests, wet preps, and slide agglutinations
- Throwing contaminated items into biohazardous waste
- Cleaning up spills
- Use of animals;
- Use of sharps;
- Production of large volumes or concentrations of potential pathogens;
- Improperly used or maintained equipment;
Examples of possible hazards are decreased dexterity or reaction time for workers wearing gloves, reduced ability to breathe when wearing N95 respirators, or improperly fitting personal protective equipment (PPE).
- Working alone in the laboratory.
No inherent biologic danger exists to a person working alone in the laboratory; however, the supervisor is responsible for knowing if and when a person is assigned to work alone. Because assigning a person to work alone is a facility-specific decision, a risk assessment should be conducted that accounts for all safety considerations, including type of work, physical safety, laboratory security, emergency response, potential exposure or injury, and other laboratory-specific issues.

2.1.3. Step 3. Consider the competencies and experience of laboratory personnel.

- Age (younger or inexperienced employees might be at higher risk);
- Genetic predisposition and nutritional deficiencies, immune/medical status (e.g., underlying illness, receipt of immunosuppressive drugs, chronic respiratory conditions, pregnancy, nonintact skin, allergies, receipt of medication known to reduce dexterity or reaction time);
- Education, training, experience, competence;
- Stress, fatigue, mental status, excessive workload;
- Perception, attitude, adherence to safety precautions; and
- The most common routes of exposure or entry into the body (i.e., skin, mucous membranes, lungs, and mouth) (Table 1).

2.1.4. Step 4. Evaluate and prioritize risks.

Risks are evaluated according to the likelihood of occurrence and severity of consequences (Table 2).

- Likelihood of occurrence
 - Almost certain: expected to occur
 - Likely: could happen sometime
 - Moderate: could happen but not likely
 - Unlikely: could happen but rare
 - Rare: could happen, but probably never will
- Severity of consequences
Consequences may depend on duration and frequency of exposure and on availability of vaccine and appropriate treatment. Following are examples of consequences for individual workers.
 - Colonization leading to a carrier state
 - Asymptomatic infection
 - Toxicity, oncogenicity, allergenicity
 - Infection, acute or chronic
 - Illness, medical treatment
 - Disease and sequelae
 - Death

TABLE 2. Risk prioritization of selected routine laboratory tasks

Task or activity	Potential hazard	Exposure risk		
		Likelihood	Consequence	Risk rating
Subculturing blood culture bottle	Needle stick — percutaneous inoculation	Likely	Infection; medical treatment	High
	Aerosols — inhalation	Moderate	Infection; medical treatment	Medium
Centrifugation	Splash — direct contact with mucous membranes	Moderate	Infection; medical treatment	High
	Aerosols — inhalation	Likely	Infection; medical treatment	High
Performing Gram stain	Aerosols from flaming slides	Moderate	Colonization; infection	Moderate
Preparing AFB smear only	Aerosols from sputum or slide preparation	Likely	Illness; medical treatment; disease	High
Performing catalase testing	Aerosols — mucous membrane exposure	Unlikely	Colonization; infection	Low
AFB culture work-up	Aerosols — inhalation	Likely	Illness; medical treatment; disease	High

Abbreviation: AFB = acid-fast bacillus.

2.1.5. Step 5. Develop, implement, and evaluate controls to minimize the risk for exposure.

- Engineering controls
 - If possible, first isolate and contain the hazard at its source.
 - Primary containment: BSC, sharps containers, centrifuge safety cups, splash guards, safer sharps (e.g., autoretracting needle/syringe combinations, disposable scalpels), and pipette aids
 - Secondary containment: building design features (e.g., directional airflow or negative air pressure, hand washing sinks, closed doors, double door entry)
- Administrative and work practice controls
 - Strict adherence to standard and special microbiological practices (*I*)
 - Adherence to signs and standard operating procedures
 - Frequently washing hands
 - Wearing PPE only in the work area
 - Minimizing aerosols
 - Prohibiting eating, drinking, smoking, chewing gum
 - Limiting use of needles and sharps, and banning recapping of needles
 - Minimizing splatter (e.g., by using lab “diapers” on bench surfaces, covering tubes with gauze when opening)
 - Monitoring appropriate use of housekeeping, decontamination, and disposal procedures
- Implementing “clean” to “dirty” work flow
- Following recommendations for medical surveillance and occupational health, immunizations, incident reporting, first aid, postexposure prophylaxis
- Training
- Implementing emergency response procedures
- PPE (as a last resort in providing a barrier to the hazard)
 - Gloves for handling all potentially contaminated materials, containers, equipment, or surfaces
 - Face protection (face shields, splash goggles worn with masks, masks with built-in eye shield) if BSCs or splash guards are not available. Face protection, however, does not adequately replace a BSC. At BSL-2 and above, a BSC or similar containment device is required for procedures with splash or aerosol potential (Table 3).
 - Laboratory coats and gowns to prevent exposure of street clothing, and gloves or bandages to protect nonintact skin
 - Additional respiratory protection if warranted by risk assessment
- Job safety analysis

One way to initiate a risk assessment is to conduct a job safety analysis for procedures, tasks, or activities performed at each workstation or specific laboratory by listing the steps involved in a specific protocol and the hazards

TABLE 3. Example of job safety analysis for laboratorians working in diagnostic laboratories: hazards and controls

Task or activity	Hazards and recommended controls			
	Potential hazard	Engineering controls	Administrative/work practices	PPE
Subculturing blood culture bottle	Needle stick—percutaneous inoculation	Safer sharps; retractable needles; puncture-resistant sharps container	No recapping; immediate disposal into sharps container	Gloves; gown or lab coat
	Aerosols—inhaleation	BSC or splash shield	Work inside BSC or behind splash shield	Face protection if not in BSC; gloves; gown or lab coat with knit cuffs
	Splash—direct contact with mucous membranes	BSC or splash shield	Work inside BSC or behind splash shield	Face protection if not in BSC; gloves; gown or lab coat
Centrifugation	Aerosols—inhaleation	BSC; removable rotors; safety cups; O-rings on buckets; plastic tubes; splash shield	Spin in BSC, or load and unload rotor in BSC; check O-rings and tubes for wear; no glass tubes; wait for centrifuge to stop before opening	Face protection if not in BSC; gloves; gown or lab coat with knit cuffs
Performing Gram stain	Aerosols from flaming slides	Slide warmer	Air dry or use slide warmer	Lab coat; gloves (optional)
Preparing AFB smear only	Aerosols from sputum or slide prep	Work in BSC; sputum decontaminant; slide warmer	Use slide warmer in BSC; dispose of slide in tuberculocidal disinfectant	Lab coat; gloves
Catalase testing	Aerosols—mucous membrane exposure	BSC; disposable tube	Work in BSC or perform in disposable tube	Lab coat; gloves; eye protection
AFB culture work-up	Aerosols—inhaleation	BSL-3 laboratory optimal; BSL-2 laboratory with BSC minimal	All work in BSC using BSL-3 practices*	Solid-front gown with cuffed sleeves; gloves; respirator if warranted

Abbreviations: PPE= personal protective equipment; BSC = biological safety cabinet; AFB = acid-fast bacillus; BSL = biosafety level.

* BSL-3 Practices include BSL-2 practice plus: restricted access; all work performed in a BSC (additional PPE); and decontamination of all waste before disposal.

associated with them and then determining the necessary controls, on the basis of organism suspected (Table 3, Appendix). Precautions beyond the standard and special practices for BSL-2 may be indicated in the following circumstances:

- Test requests for suspected *Mycobacterium tuberculosis* or other mycobacteria, filamentous fungi, bioterrorism agents, and viral hemorrhagic fevers
- Suspected high-risk organism (e.g., *Neisseria meningitidis*)
- Work with animals
- Work with large volumes or highly concentrated cultures
- Compromised immune status of staff
- Training of new or inexperienced staff
- Technologist preference
- Monitoring effectiveness of controls

Risk assessment is an ongoing process that requires at least an annual review because of changes in new and emerging pathogens and in technologies and personnel.

 - Review reports of incidents, exposures, illnesses, and near-misses.
 - Identify causes and problems; make changes, provide follow-up training.
 - Conduct routine laboratory inspections.
 - Repeat risk assessment routinely.

2.2. Principles of Biosafety (1)

2.2.1. Containment

“Containment” describes safe methods for managing infectious materials in the laboratory to reduce or eliminate exposure of laboratory workers, other persons, and the environment.

- Primary containment protects personnel and the immediate laboratory environment and is provided by good microbiological technique and use of appropriate safety equipment.
- Secondary containment protects the environment external to the laboratory and is provided by facility design and construction.

2.2.2. Biosafety Levels (Table 4)

BSLs provide appropriate levels of containment needed for the operations performed, the documented or suspected routes of transmission of the infectious agent, and the laboratory function or activities. The four BSLs, designated 1–4, are based on combinations of laboratory practice and techniques, safety equipment (primary barriers), and laboratory facilities (secondary barriers). Each BSL builds on the previous level to provide additional containment. Laboratory directors are responsible for determining which BSL is appropriate for work in their specific laboratories.

- BSL-1 is appropriate for work with agents not known to consistently cause disease in healthy human adults (i.e., laboratories that do not work with disease-causing agents or specimens from humans or animals).

TABLE 4. Summary of recommended biosafety levels (BSL) for infectious agents

BSL	Agents	Practices	Primary barriers and safety equipment	Secondary barriers (facilities)
1	Not known to consistently cause diseases in healthy adults	Standard microbiological practices	None required	Laboratory bench and sink required
2	<ul style="list-style-type: none"> • Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure 	BSL-1 practice plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • “Sharps” precautions • Biosafety manual defining any needed waste contamination or medical surveillance policies 	Primary barriers: <ul style="list-style-type: none"> • Class I or II BSC or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPE: <ul style="list-style-type: none"> • Protective laboratory clothing; gloves; respiratory protection as needed 	BSL-1 plus: <ul style="list-style-type: none"> • Autoclave available
3	<ul style="list-style-type: none"> • Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences 	BSL-2 practice plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Obtaining baseline serum from staff 	Primary barriers: <ul style="list-style-type: none"> • Class I or II BSC or other physical containment devices used for all open manipulation of agents PPE: <ul style="list-style-type: none"> • Protective laboratory clothing; gloves; respiratory protection as needed 	BSL-2 plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory

Abbreviation: BSC = biological safety cabinet; PPE = personal protective equipment.

- BSL-2 is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or by percutaneous or mucous membrane exposure (i.e., human and animal clinical diagnostic laboratories).
- BSL-3 is appropriate for work with indigenous or exotic agents that have a known potential for aerosol transmission and for agents that can cause serious and potentially fatal infections (e.g., tuberculosis laboratories).
- BSL-4 is reserved for work with exotic agents that pose a high individual risk for life-threatening disease by infectious aerosols and for which no treatment is available (e.g., laboratories working with Ebola, Marburg, and pox viruses). These high-containment laboratories have complex and advanced facility requirements.

2.3. Material Safety Data Sheets for Organisms and Chemicals

Material Safety Data Sheets (MSDS) for chemicals are available from the manufacturer, supplier, or an official Internet site. The Division of Occupational Health and Safety, National Institutes of Health, has promulgated guidelines for handling genetically manipulated organisms and has additional instructions for accessing MSDS (http://dohs.ors.od.nih.gov/material_safety_data_main.htm).

2.4. Biosafety Manual

- The laboratory director is responsible for ensuring that a laboratory-specific biosafety manual is developed, adopted, annually reviewed, and accessible to all laboratory

personnel. All laboratory employees must read this manual, and the director must maintain records of personnel who have read it.

- The manual should be reviewed and updated annually and whenever procedures or policies change. Annual training in biosafety practices is recommended for all personnel who access the laboratory. Recommended topics include the following.
 - Institutional and laboratory safety policies
 - Management, supervisor, and personnel responsibilities
 - Regulations and recommended guidelines
 - Routes of exposure in the laboratory
 - Risk assessment and reporting of exposures
 - Biosafety principles and practices
 - Standard precautions for safe handling of infectious materials
 - Standard operating procedures
 - Hazard communication and biohazard signs
 - Engineering controls
 - Administrative and work practice controls
 - PPE
 - When and how to work in a BSC
 - Transport of biohazardous materials
 - Emergency procedures
 - Decontamination and disposal of biohazardous waste
 - Training program and documentation
 - Medical surveillance and exposure evaluation procedures