Guidelines for Safe Work Practices
in Human and Animal Clinical Diagnostic Laboratories

Editor: J. Michael Miller, Ph.D., D(ABMM), F(AAM)

Writing team: Rex Astles, Timothy Baszler, Kimberle Chapin, Roberta Carey, Lynne Garcia, Larry Gray, Davise Larone, Michael Miller, Michael Pentella, Ann Pollock, Daniel S. Shapiro, Elizabeth Weirich, and Danny Wiedbrauk.
We acknowledge the assistance of: (this page is not yet complete)

Veterinarians: Dr. Larry Thompson, veterinary toxicology; Dr. Tanya Graham, veterinary pathology; Dr. Ross Graham, veterinary virology.

Corrine Fantz, PhD, D(ABCC)
Emory University
1364 Clifton Road, NE
F1476C
Atlanta, GA 30322

Thomas Burgess, PhD, D(ABCC), FACB
Quest Diagnostics
1777 Montreal Circle
Tucker, GA 30084
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1 A Culture of Safety for Diagnostic Laboratories

This document focuses on recommended biosafety practices specifically for human and animal clinical diagnostic laboratories, and is intended to be a supplement to the 5th edition of *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) developed by the Centers for Disease Control and Prevention and the National Institutes of Health (1). The BMBL was not written to address the day-to-day operation of the average diagnostic laboratory although the major principles of biosafety described remain relevant. This document is not intended to replace existing biosafety guidelines, but rather (a) to enhance the safety of activities of workers in clinical diagnostic laboratories, (b) to prompt these workers to think of laboratory safety issues that they might not have considered or addressed in the past, and (c) to encourage laboratorians to create and foster a culture of safety in their laboratories. The Clinical and Laboratory Standards Institute (CLSI) also provides further support for laboratory safety (2).

Working in a diagnostic laboratory entails safety considerations beyond the biological component; therefore this guideline addresses a few of the more important day-to-day safety issues that impact laboratorians in a setting where biological safety is a major focus.

According to the U.S. Bureau of Labor Statistics in 2008 there were about 328,000 medical laboratory technicians and technologists working in human diagnostic laboratories in the U.S. One might estimate about 500,000 total workers for both human and animal diagnostic laboratories in all professions. Many have chronic medical conditions or receive immunosuppressive therapy putting them at high risk for a
laboratory acquired infection (LAI) following a laboratory accident. Precise risk of infection after exposure is unknown since it is often difficult to determine the source or the mode of transmission. No national surveillance system is available.

The Morbidity and Mortality Weekly Report (MMWR) has documented many LAIs and examples include:

- 5 cases of laboratory-acquired Vaccinia exposures and infections --- United States, 2005-2007 (Laboratory-Acquired Brucellosis --- Indiana and Minnesota, 2006 (April 18, 2008 / 57(15);401-404)

- Laboratory-Acquired Brucellosis --- Indiana and Minnesota, 2006. (January 18, 2008 / 57(02);39-42)

- Update: Potential Exposures to Attenuated Vaccine Strain *Brucella abortus* RB51 During a Laboratory Proficiency Test --- United States and Canada, 2007 (January 18, 2008 / 57(02);36-39)

- Laboratory Exposure to *Burkholderia pseudomallei* --- Los Angeles, California, 2003 (October 29, 2004 / 53(42);988-990)

- Laboratory-Acquired Meningococcal Disease --- United States, 2000 February 22, 2002 / 51(07);141-4 (February 22, 2002 / 51(07);141-4

- Suspected Cutaneous Anthrax in a Laboratory Worker --- Texas, 2002. (April 5, 2002 / 51(13);279-281)

- Laboratory-Acquired West Nile Virus Infections --- United States, 2002 (December 20, 2002 / 51(50);1133-1135)
While bacteria account for >40% of infections with over 37 species reported as etiologic agents in LAIs, other microbes are clearly implicated:

- Hepatitis B is the most frequent of viral lab infections with a rate of 3.5 – 4.6 per 1000 workers, which is 2-4X the general population. Laboratorians who work in phlebotomys, chemistry, blood bank, hematology are vulnerable (J Clin Microbiol21:486-89. 1985).

- Early surveys of LAIs found that laboratory personnel were 3-9 times more likely to get infected with tuberculosis than the general population. (Br.Med.J 1:759-62. 1976; Br.Med J.2:10-14.1957)


- In a 1994-95 survey of 25,000 laboratory workers from 397 clinical labs in the United Kingdom, the overall LAI rate was determined to be 18/100,000 employees. (Walker & Campbell. 1999. J. Clin. Pathol. 52:415-18).

- In a 2005 CDC study of bacterial meningitis in laboratorians, *Neisseria meningitidis* was shown to be a significant cause of LAIs in the U.S. The attack rate of this organism in
the general population was calculated to be 13/100,000 persons; The attack rate for persons in the general population aged 30-59 (the estimated age of the average laboratorian) was 0.3/100,000; The attack rate for microbiologists (age 30-59) was calculated to be 20/100,000. (Sejvar, J. et al. 2005. JCM 43:4811-14).

- The most common cause of laboratory acquired fungal infections is due to the dimorphic fungi *Blastomyces, Histoplasma, and Coccidioides*. (Clin Microbiol Rev 8(3); 1995 and Ann Rev Micro 33: 1979). Most were due to inhalation of conidia.

- Reported LAI’s due to parasites were due primarily to *Leishmania, Plasmodium, Toxoplasma*, Chagas Disease organism, and Trypanosomes (Clin Microbiol Rev 2001). There were 52 cases of laboratory acquired malaria of which 56% were vector-borne (from mosquitos used in research laboratories). Most healthcare workers infected were victims of needle sticks while preparing blood smears or drawing blood.

- In clinical chemistry, data from 17 New York Hospitals (Clin Chem 26(7): 1980) listed needle puncture (103 cases)*, acid or alkali spills (46), glass cuts (44)*, splash in eye (19)*, bruises, cuts (45)* as the most frequent accidents with the “*” indicating potential for infection from microbes.

- In the hematology laboratory, the major causes of accidents are likely to be exposure to blood and body fluids; needle sticks, aerosols from centrifuge or removing tube stoppers, tube breakage; or contaminated gloves.

In the non-microbiology sections of the diagnostic laboratory the primary mistake is likely to be assuming there are no infectious agents in the specimens submitted to those sections of the laboratory.
1.1 Risk

Working in a clinical diagnostic laboratory is inherently risky. Whether the patients are people or animals and whether laboratorians work in microbiology or chemistry or another part of the laboratory, the human and animal diagnostic laboratory is a challenging environment. The more that laboratorians are aware of and adhere to recommended, scientifically based safety precautions, the more that risk is lowered. The goal of a safety program is to move the risk to as close to zero as possible although zero risk is as yet unattainable as long as live organisms are manipulated. Protecting laboratorians, co-workers, patients, our families, and the environment is the greatest safety concern, and it is the purpose of this document to focus attention to biosafety guidelines and recommendations specifically for clinical diagnostic laboratories.

For overviews of laboratory acquired infections (LAI), risk assessments, strategic principles of laboratory biosafety, and agent summary statements the reader is referred to “Biosafety in Microbiological and Biomedical Laboratories, 5\textsuperscript{th} ed” at http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

1.2 Laboratory accidents

Laboratory accidents happen! In this document “laboratory accidents” refers both to all accidents that put employees at risk of an LAI as well as actual acquisition of LAIs. Unfortunately, except for that imposed by the Select Agent Program,
there is currently no national surveillance system in place to which medical laboratory accidents and subsequent work-related infections are reported. More attention has been focused on laboratory biosafety and biosecurity since 2001, but this attention has been largely limited to the precautions required for agents of bioterrorism. Laboratory accidents and LAI’s that are not related to a bioterrorism event continue to occur, almost always because employees inadvertently stray from protocol. Because there is no official surveillance mechanism for reporting laboratory accidents and because of the fear of punitive action by some oversight agency should accidents be reported, the data needed to determine the extent and cause of LAIs is unavailable. In addition, there is a dearth of science-based insights on how to prevent LAIs. It is recommended that a voluntary, non-punitive reporting system be implemented in the United States that will allow all LAI’s to be reported and evaluated, hopefully leading to training and interventions that will facilitate a negligible incidence rate.

1.3 The five common routes of laboratory infection

The five more common routes of LAIs include:

- parenteral inoculations with syringe needles or other contaminated sharps;
- spills and splashes onto skin and mucous membranes;
- ingestion or exposure through mouth pipetting or touching mouth or eyes with fingers or contaminated objects;
- animal bites and scratches;
• and inhalation exposures to infectious aerosols. (1) The first four routes of laboratory transmission are relatively easy to detect, but they account for less than 20 percent of all reported LAIs. (3,4) No distinguishable accidents or exposure events could be identified in more than 80% of the LAIs reported before 1978 (4-6). In many cases, the only association was that the infected person worked with a microbiological agent or was in the vicinity of someone who was handling an agent. The inability to identify a specific event was also reported in the more recent study by Harding and Byers (7) who state that the probable sources of LAIs was apparent in only 50% of cases. These data suggest that unsuspected infectious aerosols can play a large role in LAIs (1;3,4,8).

1.4 A culture of safety

The concept of a “culture of safety” introduced in this document encourages all human and animal diagnostic laboratories to institute an organizational culture that embraces systematic assessment of all work processes and procedures to identify associated risks and implement plans to mitigate those risks. In addition to the often unknown biohazard risk associated with the handling of diagnostic specimens, each section of the diagnostic laboratory also has some procedures and processes with an associated degree of risk for exposure and possible infection and/or occupational injury. These risks are typically associated with design flaws and or improperly trained employees, and although present every day they are too often overlooked or ignored. The day-to-day operations of a human or animal diagnostic laboratory are quite different from that of the academic or research laboratory, for which separate guidelines exist; it is this difference that prompts
this biosafety document focusing on medical laboratory communities, their occupational risks, potential for exposure, and opportunities to mitigate those risks.

To successfully establish a culture of safety, laboratory safety needs to be an integral and apparent priority to the organization ascribed by top management and with the concomitant infrastructure support required to implement safe behaviors in its employees (10, 11). The first step in embracing a culture of safety is for the Laboratory Director to assume the responsibility to:

1. Establish and enforce a policy for a culture of safety within the laboratory.
2. Identify as many hazards as possible and specify practices and procedures that will minimize or eliminate these hazards.
3. Assure that all personnel are instructed in and engaged in performing risk assessments and demonstrate that they can identify laboratory hazards in their individual work environment
4. Assure that all personnel are trained and competent in the standard practices and techniques that minimize identified workplace hazards.
5. Provide an avenue for personnel to identify hazards and present risk mitigation strategies to management.

### 1.5 Laboratory design and architectural planning for Microbiology

Laboratory design is fundamental to the safety of laboratory workers, the hospital staff, and patients. CLSI document GP18-A2-Laboratory Design; Approved Guideline discusses laboratory design in more detail than is described here. It is difficult or impossible to remediate poorly designed laboratory workspace, so design warrants careful planning and consideration of safety issues. The following are suggestions to consider in the design or renovation of the diagnostic laboratory. Although there is no
national standard requirement for an amount of space per person working in the laboratory, one might consider 300-350 sq. ft/person as a safe figure. Ideally, it would be optimum to allow a minimum 5 foot space between the worker (at a lab chair) and any object behind the worker.

1.5.1 It is recommend that design options for the microbiology laboratory consider an enclosed component of the overall laboratory, separated by closable doors from other laboratory sections and although not a requirement, it is recommended that newly constructed diagnostic laboratories have directional inward airflow moving air from the main laboratory into the microbiology laboratory. If an open design is the choice of the facility and there is no drop ceiling, then the microbiology laboratory can have clear glass or plexiglass walls giving an open look to the laboratory but providing a safe floor to ceiling barrier from possible aerosol accidents with highly pathogenic organisms. If a drop ceiling is in place, the clear wall would need to penetrate the deck beyond the ceiling in order to seal the area. If a previously constructed laboratory does not have directional room air, the continual operation of biological safety cabinets is encouraged to provide some directionality to potential aerosols.

1.5.2 Directional air is encouraged to provide zones of containment that proceed with increasing negative pressure toward higher risk laboratory procedure work spaces. Air handling systems within the microbiology laboratory suite must be able to be adjusted and balanced with directional airflow from the corridor into the microbiology laboratory,
and then from the general microbiology laboratory into separate and enclosed tuberculosis, mycology, and virology specialty laboratories.

1.5.3 For microbiology laboratories, it is critical that the supervisor and laboratory director have significant input into the special needs of a new laboratory facility. The microbiology section must restrict access to staff only, provide a decontamination facility or have a medical waste contract in place, and provide a sink for hand washing. Hands-free sinks (foot pedal operated) are required for biosafety level (BSL)-3 facilities but not for BSL-2 facilities, although hands-free sinks are recommended installations. Benchtops must be constructed of impervious materials and not of laminate materials since these may delaminate and become difficult to disinfect. For biological safety cabinets (BSC)’s that vent to the outside, air handling should be carefully planned to ensure the air is vented to the outside after filtration, and that the outside vents are placed away from air intake units. For laboratories that contain multiple classes of BSC, it is recommended to clearly indicate to staff (by label) the hazards which are permitted to be manipulated within the specific unit (1, Appendix A). The general human and animal microbiology laboratory should be Biosafety Level 2.

For human laboratories, the separate TB and virology laboratory that manipulates cultures for identification and characterization must be Biosafety Level 3. For animal diagnostic virology laboratories in which most manipulated viruses are not human pathogens, practices should be BSL-2 unless a risk analysis indicates a high probability of a BSL-3 agent in the specimen. Risk assessments should be performed on each facility to include consideration of the specific risks encountered in each laboratory.
1.5.4 The receiving and set-up areas in microbiology should be designed with sufficient space to accommodate the greatest number of specimens that could be anticipated. This area should have a Class II BSC, a sink for hand washing, and an emergency eye wash station. Phone jacks, computer jacks, and electrical outlets should be built into the module along with refrigerator space for one or two side-by-side glass-front refrigerators or one double refrigerator to enable easy access by the set-up staff.

1.5.5 The general laboratory should have sit-down work spaces designed with adequate space for a computer at each station. It might be preferred to have work benches with storage shelves designed to sit above the center of the bench thus providing space for supplies that do not clutter the work area. Lighting should be provided under these shelves to illuminate the work area. For convenience, four electrical outlets are recommended at each work station, along with telephone and computer jacks. Gas burners are no longer universally recommended.

1.5.6 Carbon dioxide tanks and anaerobic gas tanks should be located in a space outside the actual laboratory (preferably shielded or even installed outside the walls of the building, if possible). Placing the tanks outside the laboratory or the building in a locked area will allow easy access for exchange of tanks. Lines that connect gas tanks to specific areas of the laboratory should be made of synthetic tubing to allow future moving if necessary. Accommodations should be made for daily reading of the gauges unless alarms can be installed. Gas tanks should be individually secured. (9)

1.5.7 If the laboratory plans to decontaminate its own waste prior to disposal, the laboratory must have an autoclave large enough to handle its needs. The autoclave
should be placed in a well ventilated area or be exhausted via a capture hood above it. Ideally, the mycobacteriology laboratory should have its own autoclave. Ideally, double-door autoclaves can be installed so that one side opens into the mycobacteriology laboratory and the other side opens into a disposal area used by the laboratory for disposing of other waste. Validation of the autoclave cycles for effective decontamination of the projected loads is recommended in addition to a regular maintenance and quality assurance program.

1.5.8 Optimally, the diagnostic laboratory would plan for:

1. A general microbiology laboratory area closable from the main laboratory.

2. Separate mycobacteriology and mycology rooms (under negative pressure relative to the general laboratory, Class II BSC) with phone jacks

3. Separate room for each of the following: quality control testing, supply receiving, record storage, etc.

4. An extra room for future expansion to offer more services, e.g., molecular or virology testing. The room should be able to be easily renovated to accommodate a Class II biological safety cabinet, directional air flow, phone jacks, and communication devices such as intercoms. Phone jacks and communication devices such as intercoms should be included in all such rooms.

1.5.9 Make sure current and future microbiology space is designed for an adequate number of blood culture instruments, automated identification instruments, automated enzyme immunoassay’s, nucleic acid extraction, and pipetting instruments; refrigerators; automated Gram stainers; automated plate streakers; BSCs; freezers; and additional
computer stations for optional usage. Some identification instruments require at least 8 feet of “footprint” space for the unit, printer, and modules. If the laboratory will provide the service, plan for a medium sized anaerobe chamber, about 6 ft. of footprint. Risk assessments should include evaluation of the infectious aerosols that may be produced by automated procedural equipment to determine if containment ventilation is recommended.

Resources:

1. CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, 5th edition.  

2. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from
   Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania
   19087-1898 USA, 2010. (In press)

3. Collins CH. Laboratory-acquired infections: history, incidence, causes, and prevention. Third

4. Pike RM. Laboratory-associated infections: incidence, fatalities, causes, and prevention. Annu

5. Pike RM. Laboratory-associated infections: summary and analysis of 3921 cases. Health Lab


2. **Biological risk assessment and biosafety guidelines**

2.1. Risk assessment
   - 2.1.1. Hazard identification
   - 2.1.2. Hazard control

2.2. Principles of biosafety
   - 2.2.1. Containment
   - 2.2.2. Biosafety levels

2.3. MSDS for organisms and chemicals

2.4. Biosafety manual
2. Biological Risk assessment and Biosafety Guidelines

The Laboratory Director is responsible for identifying the potential hazards, assessing the risks associated with those hazards, and establishing precautions and standard procedures to minimize employee exposure to those risks. Because the identity of the infectious agent is initially unknown in the clinical laboratory, it is a general recommendation that BSL-2 Standard and Special Practices should be followed for all work in the clinical laboratory, and Standard Precautions (gloves, gowns and protective eyewear) and BSL-2 practices should be employed when handling all blood and body fluids encountered. (1,2,3). Risk assessment, as outlined below and in section 12 regarding veterinary diagnostic laboratories, may determine that decreasing or increasing biosafety level practices or facilities is warranted.
2.1 Risk assessment

For clinical labs, the goal of a safety program is to eliminate or reduce the potential for exposure to pathogens that may be present in patient samples, and thus, reducing the occurrence of laboratory acquired infections (LAI). In its most simple terms, it consists of hazard/risk identification and hazard/risk control. There is no national standard or method to conduct a risk assessment, but consideration should include the potential agent (if known), the laboratory environment and procedures, and the personnel conducting these procedures. Standardization of the risk assessment process at an institution can greatly enhance the clarity and quality of this process. Training of staff in the risk assessment process would be a critical element in achieving these objectives.

2.1.1 Hazard identification

- **The potential agent**
  - The most common routes of transmission (ingestion via contamination from surfaces/fomites to hands and mouth; percutaneous inoculation from cuts, needle sticks, non-intact skin, or bites; direct contact with mucous membranes; and inhalation of aerosols) See Table 2.1.

  - The organisms routinely isolated, based on the specimen type, patient data (of individual or the hospital population), epidemiological data, and the geographic origin of the specimen.

  - Intrinsic factors (if agent is known).

  - Pathogenicity, virulence and strain infectivity/communicability

  - Mode of transmission (may be different in the laboratory than what occurs naturally in the environment)
• Infectious dose (The number of microorganisms required to initiate infection can vary greatly with the specific organism and the route of exposure)

• Form (stage) of the agent (e.g., presence or absence of cell wall, spore vs. vegetative state, conidia vs. hyphae for mycotic agents)

• Invasiveness of agent (ability to produce certain enzymes)

• Resistance to antibiotics
  o Indicators of possible high risk pathogens-continue work in a BSC
    • Slow growing tiny colonies at 24-48 hours and Gram stain shows gram negative rods or gram negative coccobacilli
    • Slow growth in blood culture bottles (i.e., positive at $\geq 48$ hours) and Gram stain shows small gram negative rods or gram negative coccobacilli
    • Growth only on chocolate agar
    • Rapid growth of flat non-pigmented irregular colonies with comma projections and ground glass appearance. Gram stain shows boxcar shaped Gram positive rods with or without spores.

○ The laboratory environment

○ The facility (BSL-2, BSL3, open floor plan (more risk) vs. separate areas or rooms for specific activities (less risk), sufficient space vs. crowded, workflow, equipment present, etc)
○Note: Equipment itself may be a hazard as in the case of uncertified BSC, cracked centrifuge tubes, autoclaves, overfilled sharps containers, Bunsen burners, etc.

○The laboratory procedures Specific microbiological activities that have exposure potential are listed in Table 2.1.

● Potential for generating aerosols and droplets

● Use of animals

● Use of sharps

● Producing large volumes

● Equipment

● Personal protective equipment (PPE) used

  ● Note: PPE itself can be a hazard because of decreased dexterity or reaction time when wearing gloves, reduced ability to breathe when wearing respirators, improper fitting PPE, etc.

○The personnel

● Age, sex, genetic predisposition, nutritional deficiencies

● Immune/medical status (underlying illness, receiving immunosuppressive drugs, chronic respiratory conditions, pregnancy, non-intact skin, allergies, receiving medication known to reduce dexterity or reaction time)

● Education, training, experience, competence
● Stress, fatigue, mental status, workload

● Perception, attitude, adherence to safety precautions

● The most common routes of exposure or entry into the body (skin, mucous membranes, lungs, and mouth). See Table 2.1 below.
Table 2.1. Laboratory activities associated with exposure to infectious agents (Modified from Sewell (4))

<table>
<thead>
<tr>
<th>Routes of Exposure/Transmission</th>
<th>Activities/Practices</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingestion/oral</strong></td>
<td>• Pipetting by mouth</td>
</tr>
<tr>
<td></td>
<td>• Splashing infectious material</td>
</tr>
<tr>
<td></td>
<td>• Placing contaminated material or fingers in mouth</td>
</tr>
<tr>
<td></td>
<td>• Eating, drinking, using lipstick or lip balm</td>
</tr>
<tr>
<td><strong>Percutaneous inoculation/ non-intact skin</strong></td>
<td>• Manipulating needles and syringes</td>
</tr>
<tr>
<td></td>
<td>• Handling broken glass and other sharp objects</td>
</tr>
<tr>
<td></td>
<td>• Using scalpels to cut tissue for specimen processing</td>
</tr>
<tr>
<td></td>
<td>• Waste disposal</td>
</tr>
<tr>
<td><strong>Direct contact with mucus membranes</strong></td>
<td>• Splashing or spilling infectious material into eye, mouth, nose</td>
</tr>
<tr>
<td></td>
<td>• Splashing or spilling infectious material onto intact and non-intact skin</td>
</tr>
<tr>
<td></td>
<td>• Working on contaminated surfaces</td>
</tr>
<tr>
<td></td>
<td>• Handling contaminated equipment (i.e. instrument maintenance)</td>
</tr>
<tr>
<td></td>
<td>• Inappropriate use of loops, inoculating needles, or swabs containing specimens or culture material</td>
</tr>
<tr>
<td></td>
<td>• Bites and scratches from animals and insects</td>
</tr>
<tr>
<td></td>
<td>• Waste disposal</td>
</tr>
<tr>
<td><strong>Inhalation of aerosols</strong></td>
<td>• See below</td>
</tr>
</tbody>
</table>
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
- Aspiration and transfer of 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

Manipulating specimens and cultures

- Centrifugation
- Culture set-up, inoculating media
- Mixing, blending, grinding, shaking, sonication, 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
• Preparation of isolates for automated identification/susceptibility instruments
• Preparing smears, heat fixing and staining slides
• Performing catalase test
• Performing serology, rapid antigen tests and slide agglutinations
• Throwing contaminated items into biohazardous waste

Spill clean-up

2.1.2 Hazard Control (see Table 2.2)

• Engineering controls (if possible, isolate and contain the hazard at its source first)
  ○ Primary Containment
    • BSC, sharps containers, centrifuge safety cups, splash guards, safer sharps, pipette aids
  ○ Secondary Containment
    • Building design features (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)
    • Signage and SOP’s
    • Frequent hand washing
    • Do not wear PPE outside work area
    • Minimizing aerosols
    • Prohibition of eating, drinking, smoking, chewing gum
• Limiting use of needles and sharps, no recapping of needles

• Minimization of splatter (lab “diapers” on bench surfaces, covering tubes with gauze for opening, etc.)

• Housekeeping, decontamination, and disposal procedures

• “Clean” to “Dirty” work flow

• Working in BSC if potential for aerosol generation

  ○ Medical surveillance and occupational health, immunizations, incident reporting, first aid, post-exposure prophylaxis

  ○ Training

  ○ Emergency response procedures

• PPE is reserved 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 worn with built-in eye shield) if BSCs or splash guards are not available.

  ○ Lab coats and gowns to prevent exposure of non-intact skin

  ○ Additional respiratory protection if warranted by risk assessment
One way to initiate a risk assessment is to conduct a job safety analysis (JSA) for procedures, tasks or activities performed at each workstation or specific laboratory by listing the steps involved in a specific protocol, the hazards associated with them, and then determining the necessary controls to minimize the hazard. (See Table 2.2 and Appendix 1 for examples and templates).

Table 2.2

<table>
<thead>
<tr>
<th>Task or Activity</th>
<th>Potential Hazard</th>
<th>Engineering Controls</th>
<th>Administrative/work practices</th>
<th>PPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subculture blood culture bottle</td>
<td>Needle stick-percutaneous inoculation</td>
<td>Safer sharps, retractable needles, puncture resistant sharps container</td>
<td>No recapping, immediate disposal into sharps container</td>
<td>Gloves, gown</td>
</tr>
<tr>
<td>Aerosols-inhalation</td>
<td>BSC or splash shield</td>
<td>Work inside BSC or behind splash shield</td>
<td>Face protection if not in BSC, gloves, gown</td>
<td></td>
</tr>
<tr>
<td>Splash-direct contact with mucous membranes</td>
<td>Splash shield or BSC</td>
<td>Work inside BSC or behind splash shield</td>
<td>Face protection if not in BSC, gloves, gown</td>
<td></td>
</tr>
<tr>
<td>Task or Activity</td>
<td>Potential Hazard</td>
<td>Engineering Controls</td>
<td>Administrative/work practices</td>
<td>PPE</td>
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<tr>
<td>Centrifugation</td>
<td>Aerosols-inhalation</td>
<td>BSC, removable rotors, safety cups, O-rings on buckets, plastic tubes, splash shield</td>
<td>Spin in BSC, load and unload rotor in BSC, check O-rings and tubes for wear, no glass tubes, wait for centrifuge to stop before opening</td>
<td>Lab coat, gloves, eye/face protection if not in BSC</td>
</tr>
<tr>
<td>Perform Gram stain</td>
<td>Aerosols from flaming slides</td>
<td>Slide warmer</td>
<td>Air dry or use slide warmer</td>
<td>Lab coat, gloves</td>
</tr>
<tr>
<td>Perform AFB smear only</td>
<td>Aerosols from sputum or slide prep</td>
<td>Work in BSC; sputum decontaminant Slide warmer</td>
<td>Use slide warmer in BSC; dispose of slide in tuberculocidal disinfectant</td>
<td>Lab coat, gloves, eye protection</td>
</tr>
<tr>
<td>Catalase testing</td>
<td>Aerosols, mucous membrane exposure</td>
<td>BSC, disposable tube</td>
<td>Work in BSC or perform in disposable tube</td>
<td>Lab coat, gloves, eye protection</td>
</tr>
<tr>
<td>AFB culture work-up</td>
<td>Aerosols-inhalation</td>
<td>BSL-3 laboratory with restricted access optimal, BSC minimal</td>
<td>All work in BSC using BSL-3 practices*</td>
<td>Solid front gown with cuffed sleeves, gloves, respirator if warranted</td>
</tr>
</tbody>
</table>

*BSL-3 Practices include BSL-2 practice plus:
- Restricted access
- All work performed in a BSC, additional PPE
- Decontamination of all waste before disposal

Extra 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 fever

Suspected high risk organism (*N. meningitidis*)

Work with animals

Work with large volumes or highly concentrated cultures

Compromised immune status of staff

Lack of staff training and experience

### 2.2 Principles of Biosafety (I)

#### 2.2.1 Containment

- The term “containment” is used in describing safe methods for managing infectious materials in the laboratory in order 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 the use of appropriate safety equipment
Secondary containment protects the environment external to the laboratory and is provided by the facility design and construction.

2.2.2. Biosafety levels (BSL’s). See Table 2.3

- The appropriate level of containment needed for the operations performed, the documented or suspected routes of transmission of the infectious agent, and the laboratory function or activities.
- There are four BSL’s designated 1-4, based on combinations of laboratory practice and techniques; safety equipment (primary barriers); and laboratory facilities (secondary barriers). Each BSL from BSL-1 to BSL-4 builds upon the previous level to provide additional containment. Laboratory Directors are responsible for determining what biosafety level (BSL) is appropriate for work in their specific laboratory.
  - BSL-1 is appropriate for work with agents not known to consistently cause disease in healthy human adults. These would be appropriate for school biology and science classes.
  - BSL-2 is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Human and animal clinical diagnostic laboratories should be designated as BSL-2.
  - BSL-3 is appropriate for work with indigenous or exotic agents with a known potential for aerosol transmission, and for agents that may cause serious and potentially lethal infections. Many TB labs are operated at BSL-3.
BSL-4 laboratories are reserved for exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available. These high containment laboratories have complex and advanced facility requirements. Laboratorians working with Ebola, Marburg, and pox viruses would use BSL-4 facilities.

Table 2.3 (1)

<table>
<thead>
<tr>
<th>BSL</th>
<th>AGENTS</th>
<th>PRACTICES</th>
<th>PRIMARY BARRIERS AND SAFETY EQUIPMENT</th>
<th>FACILITIES (SECONDARY BARRIERS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not known to consistently cause diseases in healthy adults</td>
<td>Standard Microbiological Practices</td>
<td>None required</td>
<td>Laboratory bench and sink required</td>
</tr>
</tbody>
</table>
| 2   | • Agents associated with human disease  
• Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure | BSL-1 practice plus:  
• Limited access  
• Biohazard warning signs  
• “Sharps” precautions  
• Biosafety manual defining any needed waste decontamination or medical surveillance policies | Primary barriers:  
• Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials  
• PPE:  
• Laboratory coat, gloves, face protection as needed | BSL-1 plus:  
• Autoclave available |
| 3   | • Indigenous or exotic agents with potential for aerosol transmission  
• Disease may have serious or lethal consequences | BSL-2 practice plus:  
• Controlled access  
• Decontamination of all waste  
• Decontamination of laboratory clothing before laundering  
• Baseline serum | Primary barriers:  
• Class I or II BSCs or other physical containment devices used for all open manipulation of agents  
• PPEs:  
• Protective laboratory clothing, gloves, respiratory protection as needed | BSL-2 plus:  
• Physical separation from access corridors  
• Self-closing, double-door access  
• Exhaust air not recirculated  
• Negative airflow into laboratory |

2.3 MSDS for organisms and chemicals

2.3.1. Material Safety Data Sheets (MSDS) for infectious substances can be found on the Public Health Agency of Canada website at:

2.3.2. MSDS for chemicals are available from the manufacturer, supplier or internet

2.4 Biosafety manual

● The Laboratory Director is responsible for ensuring that a laboratory-specific Biosafety Manual is developed, adopted and accessible to all laboratory personnel. All laboratory employees should read this manual and a record kept of those who have read it.

● This manual should be reviewed and updated annually and whenever there are procedural or policy changes. Annual training in biosafety practices is recommended for all who access the laboratory. Recommended topics include:

- Institutional and Laboratory Safety Policies

- Management, Supervisor and Personnel Responsibilities

- Regulations and Recommended Guidelines

- Routes of Exposure in the Laboratory

- Risk Assessment

- Biosafety Principles and Practices

- Standard Precautions for Safe Handling of Infectious Materials

- Standard Operating Procedures

- Hazard Communication and Biohazard Signage

- Engineering Controls

- Administrative and Work Practice Controls
- Personal Protective Equipment

- When and How to Work in a BSC

- Transport of Biohazardous Materials

- Emergency Procedures

- Biohazardous Waste Decontamination and Disposal

- Training Program and Documentation

- Medical Surveillance and Exposure Evaluation Procedures
Resources

International compendium of regulatory bodies, regulations, guidelines and resources by country, topic and subtopic:

http://www.internationalbiosafety.org/english/internlCompendium.asp

Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition:


MSDS for Infectious Substances, Public Health Agency of Canada:


American Biological Safety Association:

www.absa.org

References


(3) OSHA 29 CFR 1910.1030. Bloodborne Pathogens

3. **Fundamental Safety Practices in Diagnostic Laboratories**

3.1. Specimen receiving
   - 3.1.1. Leaking containers
   - 3.1.2. Visual contamination of the outside of containers
   - 3.1.3. Loose caps
   - 3.1.4. Operational procedures
   - 3.1.5. Manual removal of sealed caps and specimen aliquoting and pipetting
   - 3.1.6. Pneumatic tube systems

3.2. Personal precautions
   - 3.2.1. Work at the open bench
   - 3.2.2. Personal protective equipment

3.3. Biosafety cabinet

3.4. Disinfection
   - 3.4.1. Good work practices
   - 3.4.2. Bleach solutions

3.5. Waste management
   - 3.5.1. Decontamination of medical waste prior to transport and disposal
   - 3.5.2. Management of Discarded Cultures and Stocks
   - 3.5.3. Autoclave safety

3.6. Dry ice
   - 3.6.1. General information
   - 3.6.2. Disposal of dry ice

3.7. Electrical safety

3.8. Gasses in the laboratory
   - 3.8.1. Compressed gas cylinders

3.9. Liquid gasses (cryogens)
   - 3.9.1. Cold contact burns and freezing
   - 3.9.2. Asphyxiation hazards
   - 3.9.3. Explosion hazards
   - 3.9.4. Cryotube explosions
   - 3.9.5. Embrittlement
3.9.6. Infectious disease hazards

3.10. Slip, trip and fall hazards
3.10.1. Slips
3.10.2. Trips

3.11. Ultra low temperature freezers

3.12. UV light

3.13. Vacuum devices
3.13.1. Implosion safety
3.13.2. Aerosol generation
3.13.3. Vacuum devise aerosol safety
3.13.4. Disposal of liquid wastes from vacuum-assisted aspiration traps

3.14. Biological hazards
3.14.1. Preventing punctures and cuts
3.14.2. Preventing ingestion of infectious agents
3.14.3. Preventing spills and splashes onto skin and mucus membranes
3.14.4. Prevention of aerosols

3.15. Ultrasonic devices

3.16. Clean vs. dirty areas of the laboratory
3.16.1. Clean areas
3.16.2. Supervisor and laboratory director offices
3.16.3. Dirty areas

3.17. Instrumentation
3.17.1. Water baths and water pans in incubators
3.17.2. Centrifuges and cytocentrifuges
3.17.3. Automated analyzers
3.17.4. Vacuum-assisted aspiration devices
3.17.5. ELISA plate washers in microbiology
3.17.6. Identification instruments, blood culture instruments, PCR instruments

3.18. Rapid testing (kits)

3.19. Unidirectional work flow and spatial separation of work areas
3.20.
3FUNDAMENTALS OF LABORATORY SAFETY

There are many safety procedures, guidelines, and principles that apply to all sections of the diagnostic laboratory and many of those are described here. The recommendations presented in this section may be repeated in subsequent sections to provide emphasis but will generally represent a broad view of safety throughout the laboratory. Refer to other documents for more detail (1,2).

Hospitals, clinical laboratories, state and local health departments, the Centers for Disease Control and Prevention, and the American Society for Microbiology have established and/or published guidelines that should be followed when suspected agents of bioterrorism have been, or could be released in the community. However, routine clinical laboratory testing may provide the first evidence of an unexpected bioterrorism (BT) event. Routine clinical specimens may also harbor unusual or exotic infectious agents that are dangerous to amplify in culture. These agents are often difficult to identify and the routine bench technologist may continue to work up the culture by passage, repeated staining, nucleic acid testing, neutralization and other methods. This continued workup places the technologist and others in the laboratory at risk for infection. Ideally, these specimens should not be processed or tested in the routine laboratory. These specimens can be removed from the testing stream if the suspect agent is known. Relationships with the State public health laboratory and subsequently with the Laboratory Response Network are critical in this effort.

Once the testing process has begun, the bench technologists should have clear and concise instructions regarding when to seek the assistance of the laboratory supervisor and/or director.
3.1 Specimen Receiving and Log-In/Set-Up Station

- Microbiology specimens are to be received in uncontaminated containers that are intact and are consistent with laboratory specimen collection policy.
- The use of pneumatic tubes in the transport of specimens is acceptable for most specimens but may be contraindicated for specimens without sealed caps, such as urine cups; these should to be delivered by hand (see 3.1.6). Institution should adopt specific standard operating procedures (SOPs) in the event that irreplaceable specimens are considered for transportation using these systems.
- Ideally, all specimens are to be processed in a biological safety cabinet (BSC) adhering to safe BSC practices.
- Use of a four-foot wide BSC for inoculating plates and preparing smears should be limited to one employee at a time, wearing appropriate PPE. Six-foot wide BSCs may accommodate additional testing equipment or materials. Check the manufacturer’s recommendations before allowing two employees to work simultaneously in the larger cabinet.
- Minimal PPE for the general set up area is gown and gloves. In microbiology, a mask is recommended but optional. For the mycobacteriology and virology laboratory where organism manipulation is conducted, one could wear a fit-tested N-95 respirator or select other appropriate respiratory protection, based on the risk assessment. An N-95 respirator is normally not required for biocontainment levels up to BSL-2, although it provides a higher degree of protection than a surgical mask. Safe BSC practices are to
be adhered to at all times. Mycobacterial, fungal, viral, and molecular specimens may have specific additional safeguards.

3.1.1. Leaking containers

- Specimens should be submitted to the laboratory in transport bags that isolate the patient requisition from specimens and always limit one patient per bag to prevent misidentification and cross-contamination.

- Broken containers and containers that have spilled their contents are unacceptable for culture and require notification of the section supervisor. A new specimen should be requested, the incident documented, and the supervisor notified.

- Visually examine containers for leaks upon arrival and before placing on rockers, in centrifuges, in racks, in closed tube sampling (cap-piercing probe) systems, automated aliquot stations, automated slide preparation systems, or on conveyor belts.

- Track and document all incidents of cracked tubes, loose caps, and leaking containers. Increases in documented events may indicate the need to clarify or strengthen specimen acceptance policies, improve specimen collection or transportation practices, or may identify defective container lot numbers.

- Consider all sputum containers as coming from patients with tuberculosis or pneumonia and handle with care. External contamination because of inappropriate lid closure can contaminate the gloves of the laboratorian and all contents of the BSC. Change and discard gloves after cleanup. Document the external contamination for reporting purposes.

- Consider all blood culture bottles as coming from patients potentially infected (e.g. with HIV or hepatitis) and handle appropriately. If there is any concern about external
contamination, carefully disinfect the outside of the tubes or bottles prior to inserting them into the blood culture instruments. Change and discard gloves after cleanup and decontamination of the immediate area. Document the external contamination for reporting purposes.

● Leaking stool containers could possibly contain *Salmonella, Shigella*, parasites, or viral agents. Disinfect the outside of the container before culturing the contents and change and discard gloves before proceeding. Document the external contamination for reporting purposes.

● Viral specimens with damaged or leaking containers may need to be discarded before opening. Contact the supervisor and be prepared to notify the submitter, asking for another specimen.

### 3.1.2. Visual contamination of outside of container

● All specimen containers should be considered contaminated.

● Do not rely on visible external contamination to confirm potential source of contamination.

● Wipe off visible contamination using towel or gauze pad moistened with acceptable decontaminant such as a 1:10 dilution of household bleach or use the established laboratory disinfectant. Ensure label and bar code are not obscured before advancing specimen for analysis.
3.1.3. Loose caps

- Always grasp the tube or outside of the specimen container, not the stopper or cap when picking up tubes/specimen containers to prevent spills and breakage.

- Ensure tops are tightly secured on all specimen containers, blood-collection tubes, and sample tubes before advancing for analysis or storage.

3.1.4. Operational procedures

- Specimen placement, specimen flow, and bench operational workflow should be unidirectional and uniform for all operators to maximize effective use of engineering controls, i.e. from clean areas to dirty areas.

- Appropriate PPE should be determined based upon documented risk and hazard assessments of all the operations performed at each bench, and should be defined consistently in the same location in all procedure manuals and clearly posted for each operation carried out at the bench.

- Workstation procedure manuals should include instructions for the organization of all instruments, materials, and supplies in each area and include any cleaning and disinfection instructions and the frequency of cleaning and disinfection for all surfaces and instruments.

- Supervisors should routinely inspect for cleanliness of the bench.

- Have written procedures for non-laboratory operations, e.g. technical instrument maintenance, in-house or contracted maintenance, emergency response, housekeeping.
and construction and utility operations to mitigate exposures associated with assigned operational tasks.

- Write non-laboratory operation procedures for both bench operators and the non-laboratory service providers with their input and consultation.
- Document the training and assess the competency of service providers and bench operators for all written non-laboratory operational bench procedures.
- General bacteriology stains
  - Gram stain
    - Crystal violet, methylene blue, potassium iodide, and ethanol are all irritants; crystal violet is also carcinogenic and somewhat toxic; ethanol is a hepatotoxin.
    - Eye protection (safety glasses or chemical splash goggles) and disposable gloves are recommended when staining or preparing stains.
    - Risks include handling live organisms in concentrated amounts, the potential for creating aerosols, the potential for skin contamination and environmental contamination.
    - Contaminated waste should be placed in a biohazard bag for disposal.
  - All other stains, including fluorescent conjugates – refer to the MSDS sheets associated with each stain or chemical.

3.1.5. Manual removal of sealed caps and specimen aliquotting and pipetting

- Always remove caps behind a bench fixed splash shield or wear additional PPE appropriate to protect from splashes and aerosols.
● Place a gauze pad over the cap and then slowly pry or push the cap off with an away-from-body motion. Never reuse a gauze pad since this may contribute to cross-contamination. Several manufacturers market safety devices to help remove caps from tubes as well as break open ampoules. (For example, Current Technologies - Saf De-Cap).

● Use automated or semi-automated pipettes and safety transfer devices.

3.1.6 Pneumatic Tube Systems

● Establish SOPs for the use and decontamination of the pneumatic tube system (PTS).

● Breakage or leakage of specimens when transported using a pneumatic tube system risks contamination of the transport system itself.

● Limitations on use of the PTS should be based on a complete risk/hazard assessment and should include but not be limited to:

  ○ Limitations on specimen size, volume, weight and container types (this is especially applicable to cytology specimens and certain types of urine containers) sent through the tube system.

  ○ Place all specimens sent through a pneumatic tube system in a sealed zip-lock bag.

  ○ Bags should be tested and shown to be leak-proof under the conditions present in the pneumatic tube system.
○ Requisition forms should be protected by a separate pouch or enclosed in a separate secondary bag to prevent contamination.

○ A zip-lock bag should only contain specimens from one patient.

○ Place absorbent wadding between patient bags to help absorb spills and minimize contamination to the outside of the carrier.

○ Handle contaminated pneumatic tube carriers in accordance with Standard Precautions.

○ Contaminated carriers should be disinfected with bleach solution or other disinfectant following the protocol recommended by the manufacturer and approved by the hospital’s infection control committee if the system is in use in a hospital.

    • Prepare SOP’s for both laboratory operators and the non-laboratory service providers with their input and consultation.

    • Document training and competency assessment of service providers and bench operators for pneumatic tube system maintenance and decontamination procedures.

○ Wear gloves when opening PTS carriers containing patient specimens.

○ Decontaminate the outside of tube carriers before sending back to patient care areas.

○ The hospital should establish a hot-line for immediately reporting problems with the pneumatic tube system.
○ The hospital should establish an emergency PTS shut down plan including roles and responsibilities and include the implementation of an alternative specimen transport plan.

○ Develop a system to track incidents of improperly closed carriers, cracked tubes, loose caps, and leaking containers.

  • Increases in documented events may indicate the need to clarify or strengthen pneumatic tube system use policies, improve specimen collection practices, identify defective carriers and/or container lot numbers.

○ Documented training and assessment of competency should include knowledge of the risks associated with using a pneumatic tube system and the precautions to be taken to control those risks.

3.2. Personal precautions

If engineering controls are in place to prevent splashes or sprays from occurring, the requirement for PPE may be modified based on a risk assessment and evidence of the effectiveness of the engineering control to prevent exposure from splashes or sprays.

3.2.1. Work at the open bench

• Since no two workstations are identical, written procedures for each clinical laboratory workstation should include specific work practices and work practice controls to mitigate potential exposures.
● A dedicated hand washing sink with hot water should be available in each work area to wash hands following contamination of hands or gloves with blood or other potentially infectious materials. Employees should not rely on a sink in a rest room for washing their hands following work in a technical area. Frequent hand washing is essential. Each workstation should be supplied with alcohol hand rub to facilitate frequent hand cleaning and with absorbent work pads to prevent accidental splashes. Safety glasses, splash shield, surgical mask, and gloves should be available for optional use and when necessary based on the isolate.

● In the general microbiology laboratory, masks and disposable gloves are not required away from the setup station but may be voluntarily used. If gloves are used, they can easily become contaminated during routine use therefore gloves should not be washed and reused. Discard gloves and don a new pair when leaving the workstation.

● Splash guards at workstations are recommended when working at the blood culture bench, when accessing blood bottle contents after Select Agent “rule out” testing has produced negative results, or at any station where potential splashing may occur.

● Should an accident, splash, or spill occur, regardless of how small, notify nearby workers and the supervisor.

● Sniffing of bacterial cultures growing on artificial media to detect characteristic odors may or may not increase the risk of laboratory acquired infections but there is little or no scientific evidence that defines the risk or implicates sniffing as a dangerous activity. It is prudent, however, to refrain from sniffing plates as a precaution and to reduce any risk to as low as possible. Plates suspected of containing Neisseria meningitidis, or colonies shown to be gram-negative coccobacilli should never be sniffed and should be manipulated within the biosafety cabinet.
● Open flame burners should not be used anywhere in the laboratory. Use disposable loops and needles or use electric incinerators for metal wire devices.

● Disinfectant-containing discard containers and sharps containers should be within easy reach of the work station.

● Computer keyboards located at workstations should have a protective cover that is easily cleanable and should be disinfected along with the benchtop on a routine basis, but at least at the end of the work shift.

● Blood culture bottles should be tapped behind a safety splash shield or within a biological safety cabinet. Gram negative coccobacilli from blood culture bottles should be manipulated as described above and ideally handled within a BSC. Laboratories without the ability to determine or rule out Brucella or Francisella should consider directly shipping these isolates to a reference laboratory.

● Urine remaining from culture activities can be discarded down the sink drain or into the sanitary sewer.

● Feces and other specimens such as body fluids and respiratory specimens remaining from culture activities should be discarded with medical waste and autoclaved if warranted by risk assessment.

● Tissue remaining from culture activities of BSL-3 infectious agents should be discarded into medical waste and autoclaved.

3.2.2 Personal Protective Equipment
Personal protective equipment includes any of a variety of items including gloves, laboratory coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, and goggles that are designed to protect the laboratory worker from exposure to physical, biological, and chemical hazards. Personal protective equipment is often used in combination with BSCs and other devices that contain the agents or materials being handled. In some situations where it is impractical to work in a biological safety cabinet, personal protective equipment may form the primary barrier between personnel and hazardous materials(1).

OSHA defines PPE as “appropriate” if it does not permit blood or other potentially infectious materials to pass thorough or reach the employee’s street clothes, undergarments, skin, eyes, mouth or other mucous membranes under normal conditions of use.

Sources for PPE standards:

- **Lab coats** - American Society for Testing and Materials (ASTM).
- **Hand protection** - there is no ANSI standard for gloves, but there are American Society for Testing and Materials (ASTM) standards (which is now known as ASTM International) ASTM standards for disposable gloves based on the specific type of material with which the glove is made. The Food and Drug Administration (FDA) has indicated that patient examination gloves used during patient care and vascular access procedures meet its adulteration
requirements and have a 510(k) medical device registration with this agency. OSHA recommends that selection be based upon the tasks performed and the performance and construction characteristics of the glove material. Disposable gloves must be made available in a variety of sizes to ensure that employees are able to select the size that best fits their hands. Disposable gloves should also be available in a variety different materials (e.g. nitrile, chloroprene) in the event that employees have skin sensitivity issues with either the type of glove material or the accelerants or other chemicals used in the glove manufacturing process.

- The employee medical history should be evaluated for evidence of the presence of a latex allergy, if latex gloves are in use in the laboratory.
- Using the hazard assessment for a given operation, laboratory management or an assigned safety officer or safety team should select the most appropriate glove for the task as well as establish how long it can be worn.
- Before purchasing gloves, laboratory management or an assigned safety officer or safety team should request documentation from the manufacturer that the gloves meet the appropriate test standard(s) for the hazard(s) anticipated.
- Distributing PPE’s to each employee e.g. box of gloves, eye protection, face shields, and masks helps to ensure access to appropriate PPE.

Employers are required by OSHA to train employees to know at least the following:

- When PPE is necessary.
- What PPE is necessary.
• How to properly put on, take off, adjust and wear the PPE.
• The limitations of the PPE.
• Proper care, maintenance, useful life and disposal of PPE.
• Resource: OSHA Informational Booklet Personal Protective Equipment

OSHA 3151-12R 2003.

● Eye and face protection (goggles, mask, face shield or other splatter guard) must be used
whenver a splash or spray event could occur. This includes, but is not limited to opening
containers and pipetting, manipulating, aliquoting, or testing specimens, cultures, biological
agents, or other hazardous materials outside of the biosafety cabinet.
  o If eye and face protection becomes contaminated, these devices must either be
decontaminated before reuse or disposed of with other contaminated laboratory
waste.

● Neither eyeglasses nor contact lenses are considered to be personal protective equipment.
Laboratory workers who wear contact lenses must use face protection as described above.
Prescription safety glasses with side shields should be worn in the laboratory for those who
need corrected vision. In a chemical splash, contact lenses can intensify eye damage because
the lens will hold the chemical against the eye for a longer period of time.

● Protective laboratory coats, gowns, or uniforms are recommended to prevent contamination
of personal clothing. Remove protective clothing before leaving for non-laboratory areas
(e.g., cafeteria, breakroom, administrative offices). Dispose of single use protective clothing
with other contaminated waste or deposit reusable clothing for laundering by the institution.

● Do not take laboratory clothing and other personal protective equipment home for
laundering or other uses. The employer must provide laundry service for reusable protective
laboratory coats, gowns, uniforms or scrubs that are potentially or visibly contaminated with blood or other potentially infectious materials at no cost to the employee.

3.3 Biosafety Cabinet

- The Class II-A1 or II-A2 biological safety cabinet (BSC) is best suited and recommended for the diagnostic laboratory. See Table 1 for a comparison of BSC uses (1). An excellent overview and summary of the different classes and types of BSC’s is available in Appendix A of the CDC/NIH publication *Biosafety in Microbiology and Biomedical Laboratories, 5th Edition* (BMBL-5)

- Every diagnostic microbiology laboratory should have one or more BSCs to serve as a primary means of containment for working safely with infectious organisms. The College of American Pathologists (CAP) requires a BSC in microbiology in a Phase II deficiency question for microbiology departments that handle specimens or organisms considered contagious by airborne routes. There are three basic types of BSCs that are designated as Class I, Class II, and Class III.

  - The Class I cabinet is similar to a chemical fume hood and is usually hard-ducted to the building exhaust system and it protects personnel and the room environment but is not designed to protect the product inside the cabinet. This type of cabinet could be used in the general laboratory set-up area as a second choice of cabinet.

  - For most diagnostic laboratories where chemicals and toxins will not be manipulated within the cabinet, the Class II-A2 would be appropriate and easiest to install without a hard duct to the outside. This cabinet can be used at the
specimen processing station, in the mycobacteriology laboratory, the mycology laboratory, virology laboratory, and in chemistry and hematology if needed. Air can be recirculated back into the room through HEPA filters with little risk if the cabinet is maintained properly. The A-1 or A-2 BSC in the mycobacteriology laboratory is also an option with a canopy hood (thimble connection to a building exhaust duct) and annual certification. Never hard duct the Class A hood to the building exhaust system since building airflow patterns cannot be matched to the cabinet. HEPA filters remove 99.97% of particles 0.3μm in size which includes all bacteria, viruses, and spores.

○The Class III cabinet is not for routine diagnostic laboratories and is designed for highly infectious agents and provides maximum protection as would be needed in a highly complex facility that works with high consequence agents like Ebola and monkeypox virus.

●All BSCs must be certified by trained professionals as per Annex F of ANSI/NSF Standard No. 49, at least annually and each time the unit is moved. Moving the cabinet can damage the filter at the glue joint resulting in dangerous leaks, so filter and cabinet integrity must be tested after each move.

●Proper loading of the BSC and proper access by the laboratorian is described in BMBL-5, but there are some basic rules that are important to remember:

1. Do not sweep your arms into or out of the cabinet. Move arms in and out slowly, perpendicular to the face opening.

2. Install the BSC in the lab away from walking traffic, room fans, and room doors.
3. Do not block the front grill where downflow of air is conducted or the rear grill where air is removed from the cabinet.

4. Let the blowers operate at least 4 minutes before beginning work to allow the cabinet to “purge”.

5. At the beginning and end of the day, with the blower running, disinfect all surfaces with a 1:10 solution of household bleach and remove residual bleach with 70% alcohol, or use another disinfectant appropriate for the organisms encountered.

6. Do not use open flames inside the cabinet: 1st choice – disposable loops; 2nd choice – electric furnaces;

7. To decontaminate the BSC prior to maintenance, engage a BSC certification technician to use either formaldehyde gas, hydrogen peroxide vapor, or chlorine dioxide gas when the BSC is not in use.

8. UV lamps are not required in BSCs and they are not necessary.

9. Sealed rotors or safety cups on high-speed and ultracentrifuges should be opened in a BSC.

10. Where safety cups or sealed rotors cannot be used, centrifuges should be placed in a containment device or BSC designed for this purpose.

11. Medical waste generated inside of the BSC is to be collected in bags or sharps containers which are sealed before their removal and placement in medical waste containers outside of the BSC.
<table>
<thead>
<tr>
<th>BSC Class</th>
<th>Face Velocity</th>
<th>Venting</th>
<th>Applications</th>
<th>Nonvolatile Toxic Chemicals and Radionuclides</th>
<th>Volatile Toxic Chemicals and Radionuclides</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>75</td>
<td>Outside or into the room through HEPA</td>
<td>Yes</td>
<td>When exhausted outdoors 1,2</td>
<td></td>
</tr>
<tr>
<td>II-A1</td>
<td>75</td>
<td>30% vented back into the room through HEPA or to outside through a canopy unit</td>
<td>Yes (minute amounts)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>II-A2</td>
<td>100</td>
<td>Similar to II-A1, but has 100 lfm intake air velocity</td>
<td>Yes</td>
<td>When exhausted outdoors (minute amounts) 1,2</td>
<td></td>
</tr>
<tr>
<td>II-B1</td>
<td>100</td>
<td>Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter</td>
<td>Yes</td>
<td>Yes (minute amounts) 1,2</td>
<td></td>
</tr>
<tr>
<td>II-B2</td>
<td>100</td>
<td>No recirculation; total exhaust to the outside through a HEPA filter</td>
<td>Yes</td>
<td>Yes (small amounts) 1,2</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>N/A</td>
<td>Exhaust air passes through two HEPA filters in series and is exhausted to the outside via a hard connection</td>
<td>Yes</td>
<td>Yes (small amounts) 1,2</td>
<td></td>
</tr>
</tbody>
</table>

1. Installation may require a special duct to the outside, an in-line charcoal filter, and a spark proof (explosion proof) motor and other electrical components in the cabinet. Discharge of a Class I or Class II, Type A2 cabinet into a room should not occur if volatile chemicals are used.
In no instance should the chemical concentration approach the lower explosion limits of the compounds.

- In the event that an individual who works at a BSC has an infection that may have involved material manipulated in the cabinet, such as a tuberculin skin test (TST) conversion of positive results for a TB Interferon Release Assay in someone working with *M. tuberculosis*, an evaluation must be performed that includes:

  1. Evaluation and recertification of the BSCs in which the suspect work was performed.
  2. Evaluation of procedures to ensure that the individual was using proper technique working in the BSC and, if needed, re-education of the individual on proper BSC technique.
  3. Evaluation (e.g., TST testing) of others in the laboratory who work at the same BSCs and, potentially, re-education of these individuals on proper BSC technique.

### 3.4 Disinfection

#### 3.4.1 Good work practices

- Regardless of the method, the purpose of decontamination is to protect the laboratory worker, the environment, and anyone who enters the laboratory or anyone who handles laboratory materials that have been carried out of the laboratory. *For detailed information see BMBL-5 Appendix A.*

- Instructions for disinfecting a laboratory work bench should be contained in each SOP and should include what PPE to wear, how to clean surfaces, what disinfectant to use, and
how to dispose of cleaning materials. Contact time is critical and should be a part of the instructions. The instructions should also be posted in the bench area for easy reference.

- Routinely clean environmental surfaces before setting up work areas and again before leaving work areas.

- Clean any item (e.g. timer, pen, telephone, thermometer, etc.) touched with used gloves.

- Do not use alcohols or alcohol-based solutions alone to disinfect surface areas because they evaporate readily, which significantly decreases efficacy. Use disinfectants recommended for environmental surfaces, such as EPA registered disinfectants effective against HBV, HIV, and other bloodborne pathogens, or use a 1/10 dilution of household bleach. For EPA environmental disinfectant product registration information go to: www.epa.gov/oppad001/chemregindex.htm.

- Reserve sterilants and high level disinfectants cleared by FDA for processing reusable medical devices. FDA has identified manufacturers, active ingredients and contact conditions for these products. FDA-Cleared Sterilants and High Level Disinfectants list: http://www.fda.gov/cdrh/ode/germlab.html.

- Bench surfaces, stationary racks, clay tiles, rockers, slide staining racks, water/heating baths and all trays should be cleaned whenever there is a spill. All surfaces should be cleaned at the end of each shift.

- Use of disposable liners may reduce cleaning intervals but does not replace the need to clean surface areas of equipment. The underlying bench surface should be cleaned whenever the liner is discarded.
Disposable flexible polyethylene film-backed non-skid highly absorbent surface liners are available commercially and help to prevent soak-through of most solutions, including dyes and corrosive chemicals. Always discard with medical waste after contamination.

- Allow dried blood or body fluid at least 20 minutes contact with the laboratory-specified decontaminating solution to allow permeation and easy removal (1).

  ○ Never use a knife or other instrument to scrape dried blood or body fluid from surface areas as this can generate aerosols.

3.4.2 Bleach Solutions (Sodium Hypochlorite) (3)

- Hypochlorite solutions are classified as irritants and corrosives. Bleach solution is corrosive to stainless steel and thorough rinsing must follow its use in the biosafety cabinets and stainless steel sinks to remove the residue. Do not autoclave bleach solutions.

- Chlorine solutions should never be mixed or stored with cleaning products containing ammonia, ammonium chloride, or phosphoric acid. Combining these chemicals could result in the release of a chlorine gas, which can cause nausea, eye irritation, tearing, headache, and shortness of breath. These symptoms may last for several hours. If exposed to an unpleasantly strong odor following the mixing of a chlorine solution with a cleaning product, leave the room or area immediately until the fumes have cleared completely.

- To be an effective disinfectant, working bleach solutions should contain at least 0.5% but less than 2% sodium hypochlorite. Hypochlorite concentration in household bleach
varies by manufacturer. Many (but not all) household bleach solutions contain 5.25% sodium hypochlorite and a 1:10 dilution will produce a 0.53% hypochlorite solution. Use of bleach solutions with lower hypochlorite concentrations may not provide the proper level of disinfection. *A 1:10 household bleach solution should be made fresh weekly, but a fresh daily solution may provide more confidence.*

### 3.5 Waste Management

A clinical laboratory must establish a Waste Management Plan.

- As part of an on-site waste management plan, the laboratory management or the designated safety officer or safety team should:
  
  - Establish a waste reduction or minimization program.
  
  - Identify and define all categories of waste generated by the laboratory.
  
  - For each category of waste generated, determine applicability of federal, state, and local regulations including how that category of waste will be segregated, packaged, labeled/color-coded, stored, transported and tracked within the laboratory, outside the laboratory, and outside the facility to comply with the applicable regulations.
  
  - Segregate all regulated waste to prevent access by the public or clients.
  
  - Establish a system for reporting and responding to any issues or problems regarding medical waste management.
  
  - Establish treatment and disposal processes.
    
    - Disposal of regulated waste should be by a company meeting state and local licensure requirements.
A useful reference is:

CLSI. Clinical Laboratory Waste Management; approved guideline; GP05-A2, Wayne, PA:CLSI 2002.

3.5.1 Decontamination of medical waste prior to transport and disposal

- "Infectious medical waste" is defined as waste that is capable of transmitting disease. "Regulated medical waste" is any waste contaminated with significant blood or blood products in liquid or semi-liquid form and contaminated sharps; and it is considered a higher level of risk, thus warranting regulatory provisions by state or local authorities.

- Clinical laboratories must determine the federal, state, and local laws governing their organization’s regulated medical waste and ensure that the organization is in compliance with those laws. State and/or local regulations may require:
  - Permits or registration numbers to generate medical waste.
  - Development and implementation of a waste management plan.
  - Specific recordkeeping compliance.

- Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910.1030, Occupational Exposure to Bloodborne Pathogens provides minimal requirements for labeling and packaging of blood and body fluids when transported or outside a laboratory. Information may be obtained from your local OSHA office or from the Internet website: http://www.osha.gov/.
● States’ Department of Environmental Services (or equivalent) are excellent resources for assistance in complying with state and local medical waste laws.

○ To find state laws governing medical waste, visit
  
  http://www.epa.gov/epawaste/nonhaz/industrial/medical/programs.htm

(choose the state, and then look under the “Primary Materials - Cases, Codes and Regulations.” Search the State’s “Administrative Codes” or “Statutes” for information regarding waste management.) (Note: not all states refer to the waste as “infectious medical waste,” some states may refer to the waste as “regulated medical waste,” “special waste,” etc.)

○ Laboratory management must ensure that employees understand these laws and ensure regulated medical waste is not mixed with non-medical waste in a facility.

○ Employee training and competency assessment should be documented for:

  ● Constructing and properly labeling containers for medical waste that require assembly prior to their use.

  ● Disposal of medical waste in properly labeled containers.

  ● Use of appropriate supplies - containers, appropriate plastic bags, labeling etc.

  ● Following all federal, state, and local regulations regarding waste management: Handling of medical waste, immediate disposal of medical waste, storage of medical waste, transportation of medical waste which includes any required Department of Transportation (DOT) labeling (e.g., the
Consideration of the laboratory’s biosafety level is needed when discarding cultures and stocks of infectious agents.

- Discarded cultures and stocks of organisms handled under BLS-3 physical containment are to be collected and sealed in containers which are closed, leakproof, and posted with the universal biohazard symbol and the word, “Biohazard” (i.e. *Mycobacterium tuberculosis*). The containers would subsequently need to be autoclaved on site. The use of other on-site medical waste treatment technologies can be considered if these technologies sterilize the organisms, if they have been properly validated, and if they are recognized as medical waste treatment technologies by the appropriate state environmental regulatory agency.

- Decontamination is needed of discarded cultures and stocks of organisms handled at BSL-2. If this process is done on-site remote from the microbiology department, then the discarded cultures and stocks should be placed into durable, leakproof containers which are secured when they are moved. Decontamination may be done by a medical waste treatment contractor’s facility, if the waste is placed into medical waste shipping containers and packaged in accordance with applicable regulatory standards. In order to determine if these activities can be done in a manner that minimizes possible exposures, a risk assessment should be conducted. The outcome of this assessment will determine if these wastes can be safely managed off site or if they should be managed on site.
3.5.3 Autoclave Safety

- Gravity displacement steam sterilizers (autoclaves) are frequently used in microbiology, including virology laboratories. Autoclaves generate significant heat and pressure and all users must understand and respect the associated risks.

- Personnel who operate the autoclave must be trained to properly package, load, and label materials to be autoclaved in accordance with the procedures used to validate the sterilization cycle of the unit. They must also receive training in emergency procedures.

- Do not touch the sides or back of older autoclaves; they have little or no heat shielding and may cause burns.

- Do not stack or store combustible materials (e.g., cardboard, plastic materials, etc.) or flammable liquids next to the autoclave.

- Never autoclave materials that contain toxic agents, containing corrosives (e.g. acids, bases, phenol, etc.), solvents or volatiles (e.g. ethanol, methanol, acetone, chloroform, etc.) or radioactive materials.

- Place all biomedical waste to be autoclaved in an approved, biohazard-labeled autoclave bag before autoclaving. Not all red or orange bags are capable of being autoclaved. Bags selected for use in autoclaving waste must be specifically manufactured for this purpose. Use only bags represented as being appropriate for use in autoclaves when autoclaving medical waste.

- Place all sharps such as needles, scalpels, pipettes, or broken glass into approved, leak resistant, labeled, and rigid sharps container before sterilizing.
● When decontaminating a bag of dry goods such as bench paper or paper gowns, place 100 mL of water into the autoclave bag to facilitate steam production within the bag.

● Do not overfill bags or the autoclave unit as this may interfere with the sterilization process due to poor steam circulation.

● Close autoclave bags loosely with twist ties or other means that allows steam inside.

● Do not place bags directly into the autoclave. Bags should be placed onto stainless steel or polypropylene trays for autoclaving.

● Always allow an autoclave unit to cool down before opening. Stand back and open the door slowly to allow the excess steam to escape. Open the door and allow the contents to cool before handling. Always use thick, elbow-length, heat-resistant, liquid-impervious gloves to remove hot items from the autoclave.

● After autoclaving, check the autoclave indicator tape to be sure the bars are black. If the indicator tape is not activated, resterilize the load.

● A biological indicator such as *Bacillus stearothermophilus* spore strips (or equivalent) should be used at least weekly to assure that the autoclave is performing properly.

### 3.6 Dry ice

#### 3.6.1 General information

Note: Under certain circumstances, dry ice can be an explosion hazard! Dry ice is solidified carbon dioxide (CO₂) and it is extremely cold (-109°F, -79°C). Unlike water-ice, dry ice sublimates (changes directly from solid to gas) as it warms, releasing carbon dioxide gas. Carbon dioxide vapor is substantially heavier than air and in confined, poorly ventilated spaces it can displace air, causing asphyxiation.
Avoid contact with skin and eyes. Dry ice can cause severe frostbite within seconds of direct contact.

Never place dry ice into glass or sealed containers. Storage in a sealed container can result in a rupture or explosion of the container from over-pressurization.

Never handle dry ice with bare hands. Always wear insulated gloves and safety glasses. Lab coats are also recommended. Use tongs to handle blocks of dry ice. Use scoops to move pelletized dry ice.

Do not put dry ice into the mouth or otherwise ingest it. If dry ice is accidentally ingested, it can cause severe internal injury. Never put dry ice in beverages to cool them.

When transporting dry ice, place the container in the trunk of the car or truck bed and leave the car windows open for fresh air circulation. Never leave dry ice in a parked passenger vehicle. Sublimation of dry ice in a closed passenger vehicle can result in the accumulation of dangerous concentrations of asphyxiating carbon dioxide vapor. When opening a closed cargo area containing dry ice, allow the closed space to ventilate for 5 minutes before entering.

Do not place dry ice directly on bench tops, tile, laminated countertops or ceramic sinks. Use an insulating barrier such as double thickness cardboard or wood. Dry ice can destroy the bonding agent holding the tile or laminated material in place. Dry ice can also cause the bench tops and ceramic sinks to crack.

3.6.2 Disposal of dry ice

Allow the dry ice to sublimate or evaporate to the atmosphere in a well-ventilated area where no build-up of carbon dioxide vapor can occur.
• Do not dispose of dry ice in sewers, sinks or toilets. The extreme cold can fracture ceramic fixtures, crack PVC piping, and if flushed down the plumbing, the gas buildup can cause an explosion.

• Do not place dry ice in trash cans or similar containers. The extreme cold and resulting condensation can destroy these receptacles.

3.7 Electrical Safety

Electrical hazards can be divided into two main categories: those that can result in an electrical shock and those that can cause fires and/or explosions.

● Electrical shocks can be avoided by making sure that equipment and electrical cords and plugs are in good repair, grounded outlets are used, and ground-fault interrupt outlets or circuit breakers are used near sinks, eyewashes, emergency showers, or other water sources.

● Electrical circuits should not be overloaded and the use of multi-outlet power strips should be discouraged whenever possible. When power strips are necessary, their use should be approved by the Safety Department or a licensed electrician before being placed into use.

● Equipment attached to high voltage or high amperage power sources should be disconnected from the source or a lockout device should be placed on the breaker box to prevent circuit activation before maintenance is performed.

● Electrical devices could generate sparks and should not be used near flammable or volatile gasses or liquids.

● Never place flammable liquids in a household refrigerator. The spark generated by the door-activated light switch can ignite fumes trapped in the unit causing an explosion and fire.

● Specialized refrigerators must be used when storing chemicals that have explosion potential.
3.8 Gases in the laboratory

3.8.1 Compressed Gas Cylinders

● Compressed CO₂ cylinders are often used to provide gases for CO₂ incubators, and the risks associated with these incubators are minimal as long as the room is well ventilated.

● Gas cylinders represent three major safety hazards:
  ○ Gas cylinders are heavy and a falling cylinder can cause injury.
  ○ The valve attached to the cylinder is relatively fragile compared to the cylinder and if broken off, the cylinder can become a dangerous projectile.
  ○ Faulty valves or regulators may leak allowing toxic or flammable gasses to enter the room.

● In the electron microscopy laboratory, nitrogen is used to bring vacuum chambers to atmospheric pressure and critical point driers use CO₂ as a transitional fluid in the drying process for scanning electron microscopy specimens.

● Argon is used in sputter coaters, and some laboratories carry out plasma ashing of biological specimens which requires oxygen (4).

● Many of these potential hazards can be minimized by adopting safe handling practices.
○ Cylinders must be securely anchored to the wall with chains or straps to prevent falling. Cylinders less than 18 inches tall may be secured in approved stands or wall brackets.

○ When installing a new cylinder, the protective valve cap should be left in place until the cylinder is secured. The protective valve cap should be replaced before the straps or chains are removed from the cylinder.

○ Special regulators and threading are designed for each gas type. Do not try to force the threads or use the wrong regulator on a tank.

○ Regulators are normally supplied with instructions for routine maintenance and periodic checking to insure safe operation. These instructions should be followed and checks should be performed.

○ Cylinders should not be dragged, rolled, or physically carried. Do not pick cylinders up by the cap. Always use specially designed cylinder carts when moving cylinders. Cylinders must be secured to the cart and the valve covers must be attached when moving them.

### 3.9 Liquid Gases (Cryogens)

- Cryogenic liquids are liquefied gases that have a normal boiling point below -150°C (-238°F). Liquid nitrogen is used in the microbiology laboratory to freeze and preserve cells and virus stocks.
- The electron microscopy laboratory frequently uses liquid nitrogen and some laboratories also use liquid helium.
● The principle hazards associated with handling cryogenic fluids include cold contact burns and freezing, asphyxiation, explosion, and material embrittlement.

### 3.9.1 Cold Contact Burns and Freezing

- Liquid nitrogen is cold (-196°C; -320°F) and contact with liquid or gas can immediately cause frostbite. At -268°C (-450°F), liquid helium is cold enough to solidify atmospheric air.

- Always wear eye protection (face shield over safety goggles). The eyes are extremely sensitive to freezing and liquid nitrogen or liquid nitrogen vapors can cause eye damage.

- Do not allow any unprotected skin to come into contact with uninsulated piping, hoses, tongs, spargers, or other metal objects because they become extremely cold when exposed to liquid nitrogen. The skin will stick to the metal and the flesh will tear when one attempts to withdraw from it.

- Long-sleeved shirts or laboratory coats, long trousers (preferably without cuffs which could trap the liquid), closed shoes (never sandals or open shoes), and insulated cryogloves, labeled as appropriate for use with cryogenic liquids, should be worn when filling cryogenic dewars. Do not tuck pant legs into shoes or boots as this could direct liquid into the foot coverings and trap the cryogenic liquid against the skin.

- Loose fitting thermal gloves with elbow-length cuffs should be used when filling dewars. Gloves should be loose enough to be thrown off quickly if they come into contact with the liquid.
● Gloved hands should not be placed into liquid nitrogen or into the liquid nitrogen stream when filling dewars. Gloves are not rated for this type of exposure. Insulated gloves are designed to provide short-term protection when handling hoses, dispensers, and incidental contact with the liquid. Use special cryogenic liquid tongs when retrieving items from liquid nitrogen.

● There is a high risk of splattering and jets of liquid nitrogen can be generated when canes, canisters, and other objects that are at much higher temperatures are placed into liquid nitrogen. These activities can present a freezing hazard.

● Do not insert a hollow tube into the liquid nitrogen because liquefied gas may spurt from the tube.

3.9.2 Asphyxiation Hazards.

● Although nitrogen is nontoxic and inert, it can act as an asphyxiant by displacing the oxygen in the air to levels below that required to support life. Inhalation of nitrogen in excessive amounts can cause dizziness, nausea, vomiting, loss of consciousness, and death without warning.

● When liquid cryogens are expelled into the atmosphere at room temperature, they evaporate and expand to 700 - 800 times their liquid volume. Even small amounts of liquid can displace large amounts of oxygen gas and decrease the oxygen content of the atmosphere below a safe level, and the literature illustrates these dangers.(3-6)

● Do not store dewars or nitrogen containers in a confined space. The venting gas could displace enough oxygen to become a hazard.
If enclosed spaces must be used, oxygen monitors should be installed. Personnel should be trained to leave the area immediately if the alarm sounds. The alarm should be audible both inside and outside the room to prevent anyone from entering the room.

3.9.3 Explosion Hazards.

- Liquid gasses, even those that are considered inert, can be an explosion hazard.

- Heat flux into the cryogen is unavoidable regardless of the quality of the insulation provided. Cryogenic fluids have small latent heats and they will expand 700 to 800 times as they warm to room temperature. Therefore, even a small heat input can create large pressure increases within the vessel.

- Dewars must be moved carefully. Sloshing liquid into warmer regions of the container can cause sharp pressure rises.

- Do not drop, tip, or roll containers on their sides as this could damage the vessel and/or cause a sharp increase in internal pressure.

- Cryogenic containers are equipped with pressure relief devices designed to control the internal pressure. Cryogenic containers will periodically vent gasses. This is normal. Do not plug, remove, or tamper with any pressure relief device.

- Vents must be protected against icing and plugging. When all vents are closed, the expanding gas can cause an explosion. Vents must be maintained open at all times.

- Always use special ultra-low temperature containers to hold liquid nitrogen. Never place liquid nitrogen into domestic thermos flasks because they are not designed to
withstand the large and rapid temperature changes that occur when liquid nitrogen is placed in the vessel (4,5).

- Liquid nitrogen dewars should be filled slowly to minimize the internal stresses that occur when any material is cooled. Excessive stress could damage the vessel and cause it to fail.

- Liquid helium is cold enough to solidify atmospheric air. Only helium should be introduced or allowed to enter the helium volume of a liquid helium dewar. Precautions should be taken to prevent air from back-diffusing into the helium volume.

- Liquid nitrogen and liquid helium have boiling points below that of liquid oxygen and they are able to condense oxygen from the atmosphere. Repeated replenishment of the system can cause oxygen to accumulate as an unwanted contaminant. Similar oxygen enrichment may occur where condensed air accumulates on the exterior of cryogenic piping. An explosive situation may result if this oxygen-rich liquid is allowed to soak insulating or other materials which are not compatible with oxygen. In addition, some oils can form an explosive mixture when combined with liquid oxygen.

3.9.4 Cryotube Explosions

- Personal protective equipment should include an ANSI specification impact resistant face shield, heavy gloves, and a buttoned laboratory coat when removing cryotubes and ampules from nitrogen tanks.
● Cryotubes and glass ampules used for freezing cells and viruses, may explode without warning when they are removed from cryogenic storage. It is presumed that these tube explosions are caused by liquid nitrogen entering the tube through minute cracks. As the tube thaws, the rapidly expanding gas causes the tube to explode, scattering the contents of the tube (6).

● Whenever possible, ampules should be stored in the gaseous phase of the cryogenic dewar rather being submerged in the liquid nitrogen. An imperfectly sealed ampule will pick up less nitrogen in the gaseous phase.

● Nitrogen outgassing from an imperfectly sealed vial will sometimes produce a hissing noise before the vial explodes. The absence of hissing does not mean that the vial is safe. Cryotubes and ampoules should be placed onto gauze or paper toweling in an autoclavable heavy-walled container immediately after removal from the nitrogen tank and the lid of the heavy-walled container should be closed quickly. If an explosion occurs, the entire vessel should be autoclaved.

3.9.5 Embrittlement

● Cryogenic liquids should never be poured down the drain. Laboratory plumbing is one of many ordinary materials that become brittle at cryogenic temperatures and are easily fractured.

● Wood and other porous materials may trap oxygen at low temperatures and they will literally explode when subjected to mechanical shock (4),
3.9.6 Infectious Disease Hazards

- Liquid nitrogen can become contaminated when ampules are broken in the dewar and contaminants can be preserved in the nitrogen (7). These potentially infectious contaminants can contaminate other vials in the dewar and generate an infectious aerosol as the liquid nitrogen evaporates.

- Plastic cryotubes rated for liquid nitrogen temperatures are recommended for liquid nitrogen storage because they appear to be sturdier than glass ampules and are less likely to break in the nitrogen.

3.10 Slip, Trip, and Fall Hazards

- Slips, trips, and falls can cause a laboratory worker to drop or spill vessels containing infectious agents or dangerous chemicals. They can also lead to skin punctures and abrasions that make laboratory workers more vulnerable to laboratory acquired infections.

- Good housekeeping is the most fundamental means for reducing slips, trips and falls. Without good housekeeping, any other preventive measures such as installation of sophisticated flooring, specialty footwear or training on techniques of walking and safe falling will never be fully effective.

3.10.1 Slips

- Common causes of laboratory slips include wet or oily surfaces; loose, unanchored rugs or mats; and flooring or other walking surfaces that do not have some degree of traction in all areas.
● Water on the floor presents the major slip hazard. Any water on the floor should be cleaned up promptly to prevent slips and falls.

● Paraffin from tissue mounting and cutting can accumulate in tissue processing areas and can make the floor slick, despite regular cleaning, unless special floor care measures are taken.

● Mineral oils, mounting fluids, stainless steel cleaners, and other laboratory chemicals and/or reagents can create slip hazards if they get on the floor. These items should be cleaned up with soap and water whenever they are discovered.

● Cleaning floors with alcohols should be avoided whenever possible because alcohols will dissolve the wax on the floor creating areas that have different degrees of traction.

● Mats can also present a slip hazard if they are not properly anchored to the floor.

● Walking on paper, cardboard, or packaging materials can also present a slip hazard.

3.10.2 Trips

● Common causes of tripping include obstructed view; poor lighting; clutter in the walkway; mats or other items in the walkway, uncovered cables; open drawers or cabinets; and uneven walking surfaces. Mats and rugs are not recommended in microbiology.

● Drawers and cabinets should be closed except when they are being accessed.

● Clutter and items that protrude from kneehole spaces can injure workers as they move down aisles in the laboratory. Keep clutter to a minimum in order to prevent injuries. Make sure that boxes and other items do not protrude into aisles.
- Do not run electrical, data, or other cords across aisles or other walkways.

- The use of ergonomic anti-fatigue mats in other sections of the laboratory should be assessed by the Safety Officer or management before employing in a specific laboratory area. Some concerns to be aware of prior to use:
  
  - These mats are somewhat thick and the raised surface presents a trip hazard.
  
  - They serve as obstructions for carts and chairs and may cause them to tip.
  
  - They make it difficult to clean up spills.
  
  - They make it difficult for the custodial staff to clean and disinfect the floors.
  
  - These mats may also place custodial staff at risk if they pick up or move mats that have been inadvertently contaminated with chemicals or infectious agents.
  
  - Liquids will often wick under the mat, hiding potential contamination problems.
  
  - Mats present a trip/fall hazard (see above) that could impede egress from the laboratory in an emergency.

### 3.11 Ultra low temperature freezers

- Wear thermally resistant gloves and a lab coat when handling items stored at ultralow temperatures.
  
  - Specimens stored at ultralow temperatures are extremely cold (-70° to -85°C) and paradoxically, direct contact with the skin can cause severe burns.
3.12 Ultraviolet (UV) Light

- Short wave UV light has been used for surface disinfection in biological safety cabinets, in fluorescent microscopes, as a terminal disinfectant in some Type 1 water systems, and for visualizing nucleic acid bands in ethidium bromide stained gels.

- Exposure to short wave UV light has been linked to skin cancers, corneal scarring, and skin burns. These effects can result from direct or reflected UV light exposure. (8)

- UV lights are not required or necessary to assist in decontaminating biological safety cabinets in laboratories (1). If UV lights must be used for other reasons in biological safety cabinets, they should be monitored throughout their life by means of intensity sensors. Calibrated UVC sensors are a reliable and cost effective way to monitor UVC radiation levels in biological safety cabinets. Lights should be monitored because germicidal UV lights have an expected life around 9,000 hours.

- UV lights should never be used for decontaminating biological safety cabinets. Organisms in cracks, shadows, and on the underside of equipment are not affected by UV light treatment. In addition, the radiation and ozone produced by these lights will attack plastic and rubber items in and around the hood, shortening their lifespan. This exposure can affect mechanical pipette calibrations and other sensitive equipment functions.

- Germicidal UV irradiation for longer than 15 minutes is counterproductive because it produces no additional germicidal benefit and it accelerates equipment degradation.

- The UV lamp should never be on while an operator is working in the cabinet. Not all protective eyewear will protect laboratory workers from deleterious UV light exposure. Make sure the protective eyewear is rated for UVC protection.
● UV safety glasses must be worn when performing routine lamp maintenance or when there is potential for direct or indirect (reflected light) exposure.

● Gloves, long sleeved lab coat, and full face shield must be worn when working with UV view boxes.

● Placards stating “Caution, Ultraviolet Light, Wear Protective Eyewear” must be displayed in areas where UV light is used.

3.13 Vacuum devices

Vacuum-assisted filtration devices and side-arm suction flasks are used routinely in the general laboratory, whereas the electron microscopy laboratory uses vacuum-assisted evaporators, freeze-driers, freeze-fracture, and sputter coater units. Vacuum-assisted devices present implosion hazards and aerosol generation.

3.13.1 Implosion Safety

● Implosions can occur when the pressure differential exceeds the specifications of the vessel.

● Implosions can scatter sharp glass debris in all directions and seriously injure anyone in the vicinity (4,5).

● Implosions will also disperse any infectious agents that are present in the vessel.
Heavy-walled side arm suction flasks are generally rated to withstand a pressure differential of one atmosphere (14.7 PSI) and house vacuum systems or vacuum pumps that provide pressure differentials exceeding that level must be regulated with the use of an in-line pressure regulator.

Cracks, chips, and scratches in vacuum-flasks and bell jars can weaken the glass and cause an implosion even when the proper differentials are provided by pressure regulators.

Care must be taken to prevent damage to bell jars and suction flasks caused by excessive wear or impacts with hard objects.

Implosion guards made of plastic mesh or plastic boxes have been used with suction flasks to contain glass pieces if the vessel fails.

3.13.2 Aerosol Generation

Vacuum-assisted aspiration traps consist of one or two suction flasks and plumbed together in series with an in-line HEPA-filter (e.g., Vacushield™ Vent Device from Pall Life Sciences, Port Washington NY or equivalent device) to prevent contamination of the vacuum pump or house vacuum system (1).

When using a dedicated vacuum pump, many laboratories also include a suction flask containing coarse Dry-Rite (W.A. Hammond Diritre Co., Ltd, Xenia, OH) or an equivalent desiccant to remove moisture from the air, thereby protecting the pump. Aspiration traps are used in virology to remove culture media from tubes, shell vials, and other vessels prior to re-feeding or other cell manipulations. Aspiration systems are also used in ELISA plate washers.
• All of these devices generate aerosols by agitating the fluid and placing the fluid surface under reduced pressure (7). Aerosols can deposit infectious agents on the immediate surfaces and finer aerosols can be inhaled.

3.13.3 Vacuum-Device Aerosol Safety

• Aspiration devices should be used in a Biological Safety Cabinet (BSC) in order to contain any aerosols.

• Operators should wear a disposable laboratory coat and gloves to protect themselves from infectious droplets.

• When the culture aspiration is complete, the BSC blower should be run for 5 minutes to purge any airborne aerosols and the work surfaces should be decontaminated in the normal fashion.

• The in-line HEPA filters should be replaced every 6 months, when they become wet, or noticeably blocked.

3.13.4 Disposal of Liquid Wastes from Vacuum-Assisted Aspiration traps

• Never pour infectious wastes down the sink.

• Decontaminate liquid wastes from aspiration traps with bleach before disposal.
● When using an aspiration trap attached to an individual vacuum pump, laboratories usually pass the vapors through an activated charcoal trap in order to protect the pump from chlorine vapor corrosion.

● A variety of suction trap configurations are possible and the ultimate configuration will depend upon workflow and individual laboratory practice. The following procedures apply to all configurations.

○ Vacuum flasks should be changed when they are three-quarters full to prevent overfilling. Some laboratories prefer to mark the maximum fill volume on the flask and add a sufficient volume of bleach at the beginning of the day to produce a 10% bleach solution when the aspirated fluids reach the maximum fill mark.

○ Disinfect the hose by aspirating 10-50 mL of a freshly made bleach solution into the trap. Lift the hose to allow all the bleach to enter the trap. Wait 20 minutes then remove the trap from the biological safety cabinet.

○ Once decontaminated, the fluid is considered non-infectious and may be poured down the sanitary sewer.

○ Note for the virology laboratory: Bleach will reduce the phenol red dye in cell culture media and the solution will go from red to colorless. If this color change does not occur, the fluid has not been decontaminated and sufficient bleach must be added to decontaminate the vessel.

3.14 Biological Hazards

3.14.1 Preventing Punctures and Cuts
Skin punctures and cuts can directly introduce an infectious agent into the body and they can provide a route whereby a secondary agent can enter.

**Preventing Needle Sticks** (9)

- Clinical laboratories should establish a needlestick and sharps injury prevention program.

- Microtome / cryostat blades used to cut frozen sections are another potential sharp that must be handled carefully and cut resistant gloves should be worn during disassembly of the potentially contaminated blade for cleaning and disinfection.

- Limit the use of needles and syringes to procedures where there are no alternative methods. Needle-stick injuries occur most often when needles are returned to their protective sheathes after use.

- Needles should not be resheathed. If resheathing is absolutely required, the procedure should utilize a needle resheathing device to minimize injury and accidental inoculation.

- Do not use needle cutting devices because they can produce infectious aerosols. After use, place needles and syringes in leak- and puncture-resistant containers appropriately posted with the word, “Biohazard” and the universal biohazard symbol for decontamination and disposal.

- Do not bend, shear, recap, or remove needles from disposable syringes, or otherwise manipulate by hand before disposal.

**Breakage**

- Never pick up broken glass with gloved or bare hands. Use forceps, disposable plastic scoops, tongs or hemostats to pick up broken glass and dispose of the broken glass into a
sharps container. Place a broom or hand brush and dustpan in various laboratories or in the utility closet for picking up non-contaminated glassware.

- Do not use broken or chipped glassware. Discard it in the appropriate sharps container labeled for broken glassware.

- Handling broken containers with spilled infectious substances: (1)
  - Appropriate gloves are to be worn for this procedure (based on risk assessment and protection needed).
  - Cover the broken container and spilled infectious substance with a cloth or with paper towels.
  - For the routine BSL-2 laboratory, pour a disinfectant or fresh 10% household bleach over the covered area and leave for a minimum of 20 minutes. According to CDC, it would take 23 minutes to clear the air of airborne *M. tuberculosis* from a spill if the room had 12 room air changes per hour at 99% removal efficiency and 35 minutes for this removal with 99.9% efficiency. Given the variability of the number of room air changes per hour in diagnostic laboratories, the wait time should be carefully evaluated. ([http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5417a1.htm](http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5417a1.htm))
  - The cloth or paper towels and the broken material can be cleared away into biohazard sharps receptacles. Fragments of glass are to be handled with forceps, not gloved hands. (Using wadded up tape with forceps facilitates this procedure). Small HEPA vacuum cleaners are also available for removal of fine glass particulates.

- If laboratory forms or other printed or written matter are contaminated, the information on the forms or written matter are to be copied onto another form and the original is to be discarded into the biohazard waste container.
**Pasteur Pipettes**

- Whenever possible, substitute plastic or evaluate the procedure to determine if there is a newer or better technique.

- Both the top and the bottom of a Pasteur pipette can cause puncture wounds.

- Before handling glass Pasteur pipettes, examine the top of the pipette to see if it is broken or cracked. Broken pipettes can produce puncture wounds.

- When seating glass Pasteur pipettes into suction lines, hold the pipette at the top and do not allow your hand to extend below the tip. Improper technique while seating the pipette can produce puncture wounds if the hand slips or the pipette breaks.

- Dispose of used Pasteur pipettes in leak- and puncture-resistant containers.

**Other sharp devices**

- Knives, scissors, and tissue homogenizers are frequently used to dissociate tissue specimens before testing. These items must be handled carefully in order to prevent cuts and skin punctures that could injure or inoculate laboratory workers with infectious materials.

- Pointed forceps are often used for fine dissociation and for removing coverslips from shell vials. These forceps can puncture the unwary user causing injury and/or infection.

- Glass slides can break and puncture skin.

- Culture tubes, and shell vials can crack or shatter if caps are forced. The resulting shards can easily penetrate latex gloves and skin.
The lip of glass vessels may be chipped and these chipped surfaces can cut unwary laboratory workers. Chipped glassware should be discarded at the earliest opportunity.

**Sharps Disposal**

- Used disposable needles, syringes, scalpels, blades, pipettes, etc. must be carefully placed into properly labeled leak- and puncture-resistant containers used for disposal.

- Locate sharps disposal containers in or near the area where the sharps are used in order to prevent environmental contamination and injuries associated with accumulating sharps at the point of generation and from moving sharps from one place to another.

- Replace sharps containers that are 2/3 to ¾ full. Sharps containers must close securely for transport to decontamination areas. Injuries can occur when laboratory personnel try to forcibly close full containers. Once closed, over full containers can pop open, creating a hazard for other workers.

- Place non-disposable sharps into a covered leak-resistant, hard walled container for transport to a processing area for decontamination, preferably by autoclaving. (1)

- Place materials to be decontaminated off-site into a medical waste shipping container and secure for transport in accordance with applicable state, local and federal regulations (1).

- Place clean, uncontaminated sharps (e.g., clean broken glassware, chipped clean pipettes, etc.) into rigid, puncture-resistant containers for disposal in the normal trash stream. Containers should be taped shut to prevent accidental opening and potential injuries.
Never place sharp items directly into the regular trash as they could injure custodial or other staff members when the trash bags are removed from rigid trash containers.

3.14.2 Preventing Ingestion of Infectious Agents

- Refrain from touching eyes, nose, mouth, and lips while in the laboratory.
- Do not place pens, pencils, safety glasses, or other laboratory items in the mouth or against the lips.
- Do not store food or beverages for human consumption in the laboratory.
- Mouth pipetting is prohibited; mechanical pipetting devices must be used.
- Eating, drinking, smoking, handling contact lenses, and applying cosmetics is not permitted in the laboratory.
- Wash hands after working with potentially hazardous materials and before leaving the laboratory. The laboratory must have a sink for handwashing, preferably located near the laboratory exit.
- Gloves must be worn to protect hands from exposure to hazardous materials. In the molecular biology area, gloves are also used to protect the specimen from nucleases that are on the skin.
  - Change gloves when contaminated, integrity has been compromised, or when otherwise necessary.
  - Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.
  - Do not wash or reuse disposable gloves.
○ Never touch your face, mouth, eyes, or other mucus membranes when wearing gloves in the laboratory.

○ Because gloves worn in the diagnostic laboratory are considered potentially contaminated, they should be placed into biohazard disposal containers.

Remove gloves when answering the phone or using community equipment like computers.

- To prevent contamination of ungloved hands, the laboratory should be designed so that it can be easily cleaned.

○ Decontaminate work surfaces with an appropriate disinfectant after completion of work and after any spill or splash of potentially infectious material.

○ Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.

○ Chairs used in laboratory work must be covered with a non-porous material that is easily cleaned. Uncovered cloth chairs are inappropriate.

○ Carpets and rugs are not appropriate in laboratories. (1)

○ Avoid clutter in the laboratory. Boxes and papers are difficult to disinfect and may mask inadvertent spills or aerosol contamination.

○ Telephones are an effective vehicle for transferring infectious agents to the face and mucus membranes.

  • Never pick up or dial a telephone with gloved hands.
• Disinfect telephones regularly using disinfectants. Note: Alcohols do not inactivate non-enveloped viruses or destroy DNA.

• Use the hands-free or speaker phone features whenever possible to avoid touching the telephone handset to the face.

○ Never bring briefcases, purses, backpacks, books, magazines, and other personal items into the laboratory. These items are difficult to disinfect.

○ In the laboratory, computer keyboards should be considered dirty and they should be disinfected regularly. Hands should be washed after using the keyboard with ungloved hands. Keyboard covers may be used if they are removed and cleaned daily.

3.14.3 Preventing Spills and Splashes onto Skin and Mucous Membranes

● It is the responsibility of all laboratory workers to perform all procedures in a manner that minimizes the creation of splashes and/or aerosols.

● All splashes to the eye must be flushed for a minimum of 15 minutes. If a laboratory worker wearing contact lenses receives a splash to the eye, the eye must be flushed with water, the lens should be removed, and the eye should be flushed again. Disposable contact lenses should be discarded. Re-usable contact lenses should be disinfected before being returned to the eye. Manufacturer consultation may be warranted.

● Specimen containers, culture tubes, shell vials, and other cylindrical vessels used in the laboratory are easily tipped, they could roll when placed on the bench top, and glass
vessels can break if dropped. These vessels should be secured in racks whenever possible to prevent opportunities for breakage.

3.14.4 Prevention of Aerosols and Droplets

- Any procedure that imparts energy to a microbial suspension can produce infectious aerosols. (1,6)

  - Procedures and equipment frequently associated with aerosol production include pipetting, mixing with a pipette or a vortex mixer, blenders, centrifugation, and ultrasonic devices (sonicators) (1, 6, 10). These procedures and equipment generate respirable particles that remain airborne for protracted periods.

  - When inhaled, these tiny particles can be retained in the lungs.

  - These procedures and equipment also generate larger droplets that can contain larger quantities of infectious agents. The larger droplets settle out of the air rapidly, contaminating the gloved hands, work surfaces, and possibly the mucous membranes of persons performing the procedure.

  - An evaluation of the release of both respirable particles and droplets from laboratory operations determined that the respirable particles are relatively small and do not vary widely. In contrast, hand and surface contamination is substantial and varies widely. (1;11)

  - The potential risk from exposure to larger sized droplet contamination requires as much attention in a risk assessment as the respirable component of aerosols.
Aerosol Generation While Pipetting

- As noted, the pipetting action has been shown to generate aerosols, therefore it is prudent to wear gloves, eye protection, and a lab coat when pipetting and to perform pipetting operations in a biological safety cabinet.

Serological pipettes

- Pipettes and pipetting processes can be a significant source of infectious aerosols and environmental contamination.

- Culture and sample pipetting can be done safely if prudent pipetting practices are employed.

- Expelling the last drops. When the last drop of fluid is forcibly expelled out of the pipette tip, small and large droplet aerosols are formed that can contaminate the hands, and the environment (6). To minimize aerosol generation, place the pipette tip against the inside wall of tubes, flasks, or other vessels and gently expel the last drops of fluid.

- Pipette Mixing. When dispersing cell clumps, virologists frequently draw fluids into and out of the pipette to homogenize specimens and cell suspensions. A significant amount of aerosolization can occur during this process, especially when the fluid is forcibly expelled from the pipette tip. Aerosols are generated in a similar manner when “pipette mixing” culture dilutions. Closed cap vortex mixing is the preferred method for this type of mixing. If pipette mixing is required, the pipette tip should be kept below the surface of the fluid and the entire fluid volume should not be ejected from the pipette. This will reduce aerosolization and bubble formation.
Vigorous pipetting. Vigorous pipetting (rapid aspiration of fluid into the pipette) can generate aerosols within pipettes. Some, but not all of the aerosols will be trapped by the cotton plug at the proximal end of the pipette. However, some aerosols can travel through the cotton plug and contaminate the pipetting device. Some mechanical pipetting devices have HEPA filters that minimize contamination of the handset. These filters should be replaced regularly and whenever they become wet. Pipette bulbs should be decontaminated regularly and whenever they become contaminated.

Mechanical pipetters

- Hand-held mechanical pipetting devices are used for EIA testing, molecular diagnostics, and other activities that require precision.

- In molecular diagnostics, pipette contamination is the most frequent cause of false positive results (12). Pipette contamination can occur by aerosols, touching the outside of the pipette to a contaminated surface, and by contaminating the inside of the pipette during the pipetting process.

- Expelling the last remaining fluid in the tip will result in droplet splatter and aerosol formation. These aerosols and droplets can contaminate the other samples and the environment. Most mechanical pipetting devices have two stops on the plunger – the “To Deliver” stop and the “Expel” stop. Pipette volumes are calibrated for accuracy at the “To Deliver” stop and there is no need to expel the final amount of fluid in order to preserve pipetting accuracy.

- Pipette tips should be touched to the inside of the well or tube before pressing the delivery plunger. Never direct the pipetting stream into the middle of the well as this will cause splashing and contamination.
Care must be exercised when ejecting used tips into discard containers because the remaining fluid can splash and splatter widely.

The outside of the pipette barrel can become contaminated through splatter, aerosols, or by touching the barrel to contaminated objects. Do not extend the barrel of the pipette into a reagent, sample, or discard container. If normal length tips cannot reach the fluid in the tube, use extended reach pipette tips to prevent barrel contamination.

Mechanical pipetters should be disinfected regularly following the manufacturer’s instructions or with 10% household bleach followed by 70% alcohol to remove as much bleach as possible.

The use of aerosol-resistant pipette tips can significantly reduce nucleic acid contamination inside the pipette. Aerosol-resistant tips contain a hydrophobic microporous filter that is bonded onto the walls of the pipette tip. The microporous filter traps aerosols before they can contaminate the barrel of the pipette. These filters can also prevent contamination of the specimen when a contaminated pipette is inadvertently used.

**Falling drops**

When an accidental falling drop from a pipette tip encounters a hard surface, it generates aerosols and a series of small droplets, some of which may be large enough to fall and repeat the process. Greater contamination ensues when drops fall a greater distance onto a hard surface.

Many laboratories use commercial plastic-backed bench paper in BSCs and on laboratory work benches to contain or absorb this type of contamination.
● When faced with the inevitability of a falling drop, it is best to lower the tip of the pipette and allow the drop to fall a short distance onto an absorbent towel. This procedure will minimize the kinetic energy of the drop and its capacity to splatter.

**Liquid films in tubes and other vessels**

● Thin films sometimes form in the neck of culture tubes, shell vials, microcentrifuge tubes, specimen vials, and other containers. Breaking or popping this film produces aerosols and microdroplet splatter that can contain infectious agents, nucleic acids, or other potential contaminants (6).

● Containers with thin films in the neck should be re-capped and centrifuged whenever possible to disrupt the film or cause it to merge with the fluid in the vessel.

● If centrifugation is not possible (e.g., culture flasks), gauze or another absorbent material should be placed over the opening and a pipette should be inserted into the flask to disrupt the film. Dispose of the pipette and the absorbent material with other contaminated materials.

**Opening microcentrifuge tubes**

● Microcentrifuge and other plug-topped tubes will often produce aerosols and splatter when opened.
Microcentrifuge tubes should be subjected to a quick "pulse" centrifugation before they are opened in order to minimize the amount of fluid on the cap.

Microcentrifuge tubes should be opened in a biological safety cabinet whenever possible.

When opening plug-seal microcentrifuge tubes, cover the top of the tube with absorbent material (i.e. alcohol moistened gauze) to catch any splatter that might occur. Dispose of the absorbent material with other contaminated materials.

Lyophilized Materials, Serum Vials and Ampoules

- Opening vials of freeze-dried (lyophilized) material can be hazardous because these fine dry powders are easily dispersed into the atmosphere when air rushes into the evacuated vessel. The following procedure may be used to safely open a serum vial containing lyophilized material.

  - Move the vial and the suggested diluent (water or medium as appropriate) to a biological safety cabinet.

  - Wear gloves and lab coat when opening lyophilized vials.

  - Use a hemostat to remove the aluminum crimp from the vial. Discard the crimping material into the sharps container.

  - Cover the stopper with a moistened gauze pad and carefully lift the edge of the stopper and allow air to slowly enter the vial. Do not disturb the contents of the vial.
Once the vacuum has been released, remove the stopper completely and place it upside down on absorbent paper.

Add the appropriate amount of diluent to the vial using a sterile pipette.

Replace the stopper and allow the vial contents to hydrate for several minutes.

Discard the gauze, stopper, and absorbent paper with other contaminated materials.

Using a pipette, transfer the contents of the vial to an appropriate container.

Discard the original vial with other contaminated materials.

Using needles to remove infectious agents from serum vials

Needle and syringe methods for removing infectious agents from serum vials are not recommended because they can contaminate the environment and because they provide opportunities for needle-stick injuries. Use forceps, not needles, to remove serum separator tubes that are stuck in centrifuge carriers.

Glass Ampoules

Once opened, glass ampoules can be a cut and puncture hazard.

The use of safety ampoule breakers can prevent injuries by covering the ampoule during the breaking process.

For ampoules containing infectious materials, cover the score line with gauze moistened with disinfectant then break as usual using the safety ampoule breaker.
• Place the ampoule breaker into a beaker containing 10% bleach after removing the ampoule.

3.15 Ultrasonic devices

• Ultrasonic devices are principally used to lyse bacteria and viruses, and to clean glassware and laboratory equipment.

• Use the lowest effective power setting to minimize aerosol generation.

• Bath sonicators should be covered while in use.

• Articles destined for ultrasonic cleaning should be properly decontaminated prior to cleaning to prevent aerosolization of infectious agents (6).

• Organism lysis and homogenization procedures should always be performed in closed containers.

• Bath fluids should be changed frequently to prevent aerosolization of bacterial and fungal contaminants present in the bath.

3.16 Clean vs dirty areas of the laboratory

In the microbiology laboratory, all of the technical work area of the department should be considered dirty. The same concepts of demarcation and separation of molecular testing areas (see below) can be used to establish clean and dirty areas within the other sections of the diagnostic laboratory.

3.16.1 Clean areas
● Wear different color lab coats in clean vs dirty areas of the laboratory (have them available at entrance to clean areas) or require no lab coats in clean areas

● Decontaminate reusable materials and devices (such as telephone, clocks, computers, tissue boxes, work books) brought into the clean area unless known to be new and immediately apply laboratory-designated color coded tape

● A visual reminder on small objects such as workbooks, tissue boxes, and pens, can easily identify items located to a clean area.

● Demarcate separation of dirty/clean floor areas with tape (must be tape that will stand up to floor cleaning) to clearly denote clean/dirty area boundaries.

● Develop policy for cleaning and maintaining clean areas

● Train all personnel (including service personnel) how to identify and maintain clean areas and to recognize the significance of the demarcation tape and other means of area identification.

● Document training and assess competency in use of and maintaining clean areas

### 3.16.2 Supervisor and Lab Director Offices

● Offices that open into the clinical laboratory represent hybrid areas within the laboratory. These offices are not typically designed or maintained in a manner that allows for easy or efficient disinfection.

● Keep a supply of hand disinfectant gel in all office and work areas and use the gel frequently

● Components of offices that should remain clean but may be overlooked include:
Laboratory documents reports and records, small equipment, pens, procedure manuals and other items that have been in the laboratory and (could have been) handled with gloved hands.

Carpets and chairs that are difficult to disinfect

Books, journals, and other reference materials that can be taken into the lab, taken home, and/or loaned to others for use outside the laboratory

Personal items (e.g., photographs, awards, briefcases, coats, boots, backpacks, purses, personal electronic devices, etc.) that are difficult to disinfect and would not be allowed in the general laboratory.

Food items.

Designating these areas as “clean” does not necessarily make or keep them uncontaminated, especially when potentially contaminated items are brought into the office and reference materials and documents move freely between the office and laboratory. The following procedures can help to reduce the risk of contamination in laboratory office areas.

Never bring specimens, cultures, proficiency samples and similar items should into office areas.

Personal protective equipment must be removed before entering the offices and hands should be washed before entering these areas.

Establish a dedicated and protected clean area for personal items e.g. purses, briefcases, and similar items.

Disinfect desks and personal workspaces, telephones, and computer keyboards in office areas regularly.
● Refrain from touching eyes, nose, mouth, and lips while in office areas.

● Do not place pens, pencils, eyeglass bows, or other items in the mouth or against the lips.

● Do not apply or permit cosmetics in office areas.

● Do not store food in the office.

● Wash hands after working in the office and before entering common areas such as rest rooms, administrative areas, cafeteria, and the library.

● Avoid clutter in office areas as much as possible. Boxes and papers and other items make it difficult to clean and decontaminate.

● Laboratory Directors and Supervisors should assess the exposure risks associated with the use of laboratory documents and reference materials in the dirty areas of the laboratory and develop use policies to minimize those risks.

3.16.3 Dirty Areas

● All areas of the working laboratory, all equipment and keyboards, items, waste, and surfaces are considered “dirty” areas.

● There are no current standards that describe operating procedures within dirty areas of the laboratory. Laboratorians must be vigilant in recognizing the potential or risk of transmitting an etiologic agent by touch.

3.17 Instrumentation
Whether automated or manual, procedures with the potential for producing specimen aerosols and droplets (e.g. stopper removal, vortexing, opening or piercing evacuated tubes, automatic sample dispensers, etc.) require PPE and engineering controls designed to prevent exposures to infectious agents.

3.17.1 Water baths and Water (humidification) Pans in CO2 Incubators

- Clean regularly even if disinfectants are added to the water.

- To reduce bioburden, add disinfectant such as a phenolic detergent, fungicides, or algaecides, to the water as needed. Avoid using sodium azide to prevent growth of microorganisms (sodium azide forms explosive compounds with some metals).

- Raise the temperature to 90°C or higher for 30 minutes once a week for decontamination purposes

- Immediately clean after a spill or breakage

- Water baths and humidification pans in CO2 incubators can harbor bacteria, algae, and fungi that are aerosolized when the water bath lid or incubator doors are opened. These aerosols can contaminate cultures and the environment.

- Empty and clean water baths and humidification pans regularly to minimize organism buildup and the production of biofilms that are notoriously difficult to remove.

3.17.2 Centrifuges and Cytocentrifuges

- Centrifuges can be extremely dangerous instruments if not properly cleaned, maintained and correctly operated. Laboratory staff must be trained in centrifuge operation and the hazards associated with centrifugation.
Current regulations governing the manufacture of centrifuges ensure that operators are safeguarded against some potential accidents and exposures by the fitting of lid locks that prevent opening of the lid while the rotor is still spinning, imbalance detectors, devices to prevent rotor over-speed, and use of construction materials that can withstand any rotor failure. Older centrifuges without these safeguards must be operated with extreme caution and laboratories should have documented risk assessments and operating manuals that specifically provide operating instructions to mitigate these specific hazards.

Laboratories should have operating manuals for each particular centrifuge type.

Operators should have documented training and competency assessments on each particular centrifuge type they operate.

- Documented instruction for each centrifuge type should include proper instrument start-up and shutdown, emergency procedures and shut down, balancing of tubes, use of safety cups and covers, rotor and container selection, requirements for high speed and ultracentrifuges, and container fill-height limitations

All high speed and ultracentrifuges should be operated on a stable, resonance-free surface (floor, bench top, or heavy table) with at least 6-inch clearance at the sides and 4 inches at the rear of the centrifuge.

With materials handled at Biosafety Level 2 or higher levels of containment, rotors should have aerosol containment ("O-rings") and gasketed safety cups regardless of where they are used.

Rotors should be loaded and unloaded in a biosafety cabinet, particularly in virology
and mycobacteriology sections.

● Manufacturer instructions for use and care of centrifuges and especially rotors should be strictly enforced to prevent the serious hazards and potential exposures associated with rotor failure. Store rotors in a dedicated clean space and in an environment specified by the manufacturer.

● Centrifuges should be routinely cleaned at the end of each shift and immediately after a spill

● Never operate centrifuges with visible blood or body fluid spills

● Rotors should have annual stress testing and a complete certified analysis – most centrifuge manufactures offer this service

● A complete and comprehensive rotor log should be kept for every high-speed and ultracentrifuge rotor, and should include all user names, run dates, durations, speeds, total rotor revolutions, and any notes on rotor condition.

● Rotors should be retired after the manufacturers' recommended revolutions or years of service, whichever comes first, except where an annual stress test (magnaflux or other professionally recognized analysis) proves an absence of structural flaws. Long term budgetary planning for this event is important.

● During normal operations, air issues from centrifugation ventilation ports at high speeds and any infectious particles present in the airflow will be disperse rapidly and widely (10).
● Tube breakage during centrifugation presents the greatest risk for contamination as large aerosol clouds are produced. A more difficult to detect contamination can occur when centrifuging tubes without gasketed safety caps.

● The airflow rushing around the tubes can create a Venturi effect that can draw fluids from the threads of screw-capped tubes. The high velocity airflow can also aerosolize dried or liquid materials that might be present on the outside of the tube.

● Consistent use of gasketed centrifuge safety cups and sealed rotors can significantly reduce the risks associated with centrifuging infectious or potentially infectious materials.

● After centrifugation, safety cups and sealed rotors should be opened in a biological safety cabinet in order to minimize aerosol spread and environmental contamination.

● Centrifugation equipment must be properly maintained in order to prevent malfunctions and aerosols within the centrifuge.

● Centrifuge spill kit containing puncture resistant gloves, tweezers or forceps, cotton, hemostats, broom, hand brush, and dustpan should be available.

● If a specimen tube breaks within the plastic screw-capped canister or bucket in a centrifuge:

  ○ Turn the motor off and allow time for aerosols to settle before opening the centrifuge.

  ○ Remove the canister and place in a BSC.

  ○ Notify senior person in charge and other colleagues working in the area.

  ○ While wearing protective clothing, open the canister under the safety cabinet.
○Pour a 1:10 dilution of bleach or a non-corrosive disinfectant into the canister to decontaminate all surfaces; let the canister soak in bleach or disinfectant solution for 20 minutes. Clean canister thoroughly.

○Do not pick up broken glass with gloved hands. Use forceps or cotton held in forceps, or tongs or hemostats and dispose into a biosafety sharps container.

○Discard all non-sharp contaminated materials from canister into a red biohazard bag for biohazard waste disposal.

○Unbroken capped tubes should be swabbed with the same disinfectant then swabbed again, washed with water and dried.

○All materials used during the clean-up must be treated as infectious waste.

**NOTE:** If the specimen tube breaks in a centrifuge that does not have individual canisters, but does have a biohazard cover and sealed rotor, follow the manufacturer’s instructions for cleaning and decontamination.

### 3.17.3 Automated Analyzers

- Automated analyzers frequently have added features to help reduce operator exposures but do not totally eliminate potentials for exposure. A common feature in newer systems is closed system sampling.

- Sample probes that move quickly or deliver fluid rapidly may generate aerosols and droplets.

- Always use instruments according to manufacturer instructions.
● Ensure instrument safety shields and containment devices are in place at time of use.

● Limit the amount of hand movement near the sample probe and liquid-level sensors.

● Wear gloves and use gauze pads with impermeable plastic coating on one side on instruments where the operator is required to wipe sample probes after sampling.

● Newer instruments have automatic probe wash cycles eliminating this source of exposure.

● Sample trays and sample plates should be handled with caution and covered when not being sampled to prevent spillage.

● Sample cups and aliquot tubes should be filled using mechanical devices and never decanted.

● Effluents of clinical analyzers should be considered contaminated and disposal should comply with state and local regulations.

● Follow manufacturer instructions for routine cleaning and trouble-shooting specimen spills on or within an instrument, including the appropriate PPE and type of cleaning solution to be used.

● When manufacturer instructions do not include spill containment and clean up instructions, collaborate with manufacturer to develop an SOP that will effectively protect the operator and maintain and extend the instrument’s operational life.

● Safety guidelines for cell sorters have been published. See Cytometry Part A 71A: 414-437, 2007. Consider adding bleach to the waste receptacle so that a full receptacle would contain about 10% bleach.

3.17.4 Vacuum-assisted aspiration devices (see 3.13)
3.17.5 ELISA Plate Washers in microbiology

- ELISA plate washers can create aerosols and droplets by agitating the fluid and placing the fluid surface under reduced pressure. Large particle droplets generated by the washing and aspiration process can deposit infectious agents on the immediate surfaces and finer aerosols can travel greater distances and can be inhaled.

- ELISA plates should be handled with gloves at all times and should be considered to be infectious.

- ELISA plate washers and the area around the washer should be disinfected with each day of use.

- Whenever possible, the laboratory should place aerosol containment covers over ELISA plate washers to minimize aerosol contamination of the environment and laboratory workers.

3.17.6 Bacterial Identification and antimicrobial susceptibility instruments, blood culture instruments, PCR instruments, and other laboratory instruments and devices should be cleaned or disinfected according to the manufacturer’s directions or recommendations. The routine and emergency cleaning procedure for each instrument must be a part of the safety component of the procedure manual.

3.18 Rapid Tests (kits)

- Whether the rapid test is conducted in the laboratory or at the point of care, used testing kits should be considered contaminated and disposed of appropriately.
● Use of rapid testing kits should be limited to a specific area of the laboratory to maximize efficiency of environmental controls to prevent aerosol exposures when manipulating reagents, samples, and controls.

● Disposable flexible polyethylene film-backed non-skid highly absorbent surface liners are recommended to contain spills and minimize contamination of test kit materials and box.

● Outside of test kits should be wiped off with appropriate laboratory disinfectant before returning to storage area.

3.19 Unidirectional work flow and spatial separation of work:

● Reagent preparation is the cleanest area, then specimen preparation area, and finally product detection area.

● Care should be taken to leave transportable items, e.g. pens, tape, scissors, glove boxes etc. in each designated area.

● Laboratory coats and gloves should be changed and hands washed before entering each area.

● Emergency response to potentially infectious aerosol release (outside a biological safety cabinet):
  ○ All persons must immediately vacate the laboratory unit where the spill occurred.
  ○ Exposed persons are to be referred for medical advice and evaluation.
  ○ The laboratory supervisor and biosafety officer are to be informed immediately of the situation.
○ No one is to enter the room for at least 30 minutes to allow aerosols to be carried away and heavier particles to settle.

○ If the laboratory does not have a central air exhaust system, entrance is to be further delayed (e.g. up to 24 hours).

○ Signs are to be posted indicating that entry is forbidden.

○ After the appropriate time, decontamination should proceed as supervised by the biosafety officer. PPE must be worn.
Reference:


Transportation of specimens outside the lab: US Department of Transportation publication:

4. Tuberculosis laboratory safety

4.1 Specimen receiving and log-in/ set-up station
   4.1.1.1 Specimen receiving in the main microbiology laboratory
   4.1.1.2 Specimen receiving in other laboratory sections

4.1.2 Leaking containers
   4.1.2.1 Visual contamination on outside of container
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4. Tuberculosis Laboratory safety

Tuberculosis (TB) resulting from exposure to infectious aerosols remains a major risk for laboratorians. There is no safe level of exposure since exposure to as few as 1-10 organisms can cause disease. To reduce exposures to *Mycobacterium tuberculosis*, a hierarchy of controls must be employed, including: safe work practices, use of containment equipment, and specially designed laboratory facilities (1). Kubica estimated that between 8-30% of laboratorians may experience tuberculin conversions (2). Tuberculosis laboratories should be separate and isolated from the main microbiology laboratory. All policies and practices related to safety should be developed through a risk assessment process and documented in the biosafety manual.

- It is the director’s responsibility to ensure that every new employee receives safety training which should include: proper and safe handling practices; use of safety equipment such as the BSC; personal protective equipment; decontamination procedures, spill clean-up; use of the autoclave; waste disposal; tuberculosis disease symptoms; and reporting illnesses and exposures.

- Most importantly, adherence to biosafety practices must be monitored and an annual competency assessment be completed.

4.1 Specimen Receiving and Log-In/Set-Up Station
In most clinical laboratories specimens are first received in the main microbiology lab where they are logged-in and processed for other bacteriological testing. The specimens are moved to the TB laboratory for further processing specific for TB.

**Specimen receiving in the main microbiology laboratory**

- A wide variety of specimens are received for tuberculosis testing including sputum, urine, tissue, CSF and gastric washings. Specimens for tuberculosis testing can be received in the main microbiology lab, that is, a BSL-2 facility.

- At the receiving, log-in/set-up station, all clinical samples submitted for bacteriological testing including TB testing and prior to decontamination procedures, are performed in at least a BSL-2 Laboratory for non-aerosol generating activities.

- All clinical samples submitted for testing must be handled using personal protective equipment consisting of a laboratory coat and gloves. Work with these specimens is performed in a biosafety cabinet (BSC) (3) certified at least annually.

- Before opening any specimen container, disinfect the outside by wiping it with a gauze soaked in a tuberculocidal disinfectant. This action should be taken with every specimen regardless of the presence of visible contamination.

- The specimens are then moved to the tuberculosis laboratory where all procedures for TB specimen decontamination, culture propagation and
subsequent manipulation of the cultures, are performed using BSL-3 facilities, containment equipment, practices and respiratory protection (1). The BSL-3 must be properly maintained, certified, and the door to the lab kept closed.

Specimen receiving in other laboratory sections

- When processing specimens in surgical pathology suite an N-95 particulate respirator should be worn during frozen sectioning. Do not use propellant to flash-freeze tissue.

- When performing autopsy procedures, bone saws must have a vacuum attachment to minimize dispersal of bone dust. If tuberculosis is suspected, an N-95 particulate respirator should be worn during the procedure and not removed until sufficient time after the procedure for effective removal of airborne particles by the ventilation system, as indicated in the laboratory biosafety manual.

4.1.2 Leaking containers

- Collect specimens into a leak-proof container, and transport the specimen in a sealable leak-proof plastic bag. A proper container ensures that handling of the specimen can begin without external contamination.

- The transport bag is opened inside a BSC to guard against the creation of aerosols, spray and splatter.

4.1.3 Visual contamination on outside of container
• Specimens that leak during transport must be rejected and a new specimen requested. Before opening a transport bag, observe the specimen for leakage.

• If the outside of the container is grossly contaminated with the contents of the container, reject the specimen, document the rejection, and request another specimen.

• When examination of the exterior of the specimen container demonstrates minor or superficial contamination, clean the exterior with an appropriate disinfectant before further handling.

4.1.4 Loose caps

• Care should be taken when opening a specimen container since splashing or splattering may contaminate the outside of the container.

• Wipe the exterior of the container with gauze soaked in a tuberculocidal disinfectant after removing and replacing caps.

4.2 Stains and Disposal

The preparation of smears should be done in a BSC since aerosols, droplets and splatters can be generated. Unstained smears may contain viable tubercle bacilli and should be handled with caution.

4.2.1 Gram stain

Specimens submitted for routine cultures, especially sputum and other respiratory
specimens, may contain tubercle bacilli and must be handled with care regardless of whether or not AFB cultures were ordered.

4.2.2 **Acid-fast stains - Kinyoun; Ziehl-Neelsen; auromine/rhodamine**

*(fluorescent)*

- Before removing smears from the BSC, heat fix the slide on an electric slide warmer with the temperature set between 65°C and 75°C for 2 hours. Monitor and record the temperature of the slide warmer each day of use. Even after heat fixing, the slide may contain viable tubercle bacilli and should be treated as contaminated.
  - For laboratories that do not process AFB cultures but wish to make a direct smear, disinfectant (i.e. 5-6% bleach solution) may be added to the specimen to render *M. tuberculosis* non-viable and the smear made.

4.3 **Culture reading and acceptable activities at the open bench**

- Only those activities that are solely observational and do not risk the creation of aerosols can be performed at the open bench. Any manipulation of colonies of growth is performed within the biological safety cabinet (BSC) (4). Only closed, non-glass containers of culture with the outside of the container properly disinfected can be brought out of the BSC for spectrophotometer or other observational readings. However, it is preferred that all work with cultures is conducted inside a certified biological safety cabinet (BSC).
• Spill procedures must be in place to address the possibility of culture breakage (see below). Viable cultures must be transported securely using racks, safety carriers and/or carts to prevent breakage.

• Work surfaces are decontaminated each day testing is performed in the lab.

4.4 Personal precautions and work practices

Precautions and work practices are selected with regards to the potential quantity of tubercule bacilli potentially encountered in the procedure performed. Hence specimens have a lower concentration than a culture where the number of organisms is amplified. All protocols in the TB lab are evaluated for the potential to generate aerosols through the risk assessment process. Simply stated, aerosols are generated whenever energy is imparted into the specimen. Common aerosol generating procedures are: pouring liquid cultures and supernatant fluids; using fixed-volume automatic pipetters; and mixing liquid cultures with a pipette.

• Laboratorians who handle specimens from which *Mycobacterium tuberculosis* is a suspected pathogen and/or perform diagnostic testing for *M. tuberculosis* must have at least annual testing for tuberculosis infection. This can be accomplished by a Tuberculin Skin Test (TST) or Interferon Gamma Release Assay (IGRA). If the TST is performed, then a two step process is used upon hiring and followed thereafter by a one step PPD. More frequent screening for TB may be necessary if there is a laboratory accident with risk of exposure to tuberculosis or a documented conversion. A TST should not be
placed if the laboratorian has a history of either BCG vaccine or previous positive TST, in which case, an IGRA should be performed.
  o Personnel must be aware that certain changes in health, for example, receiving chemotherapy, may place them at increased risk for tuberculosis if exposure occurs.

4.4.1 Personal Protective Equipment (PPE)

- The solid front disposable gown with snug (knit) cuffs is routinely used as protection against sprays and splatter.
- Gloves are worn at all times when working in the BSL-3 laboratory and must be long enough to externally overlap the sleeves of the gown. In the BSL-2 environment, the wearing of gloves is dependent on the laboratory’s routine practice that is guided by a risk assessment.
  o In general, gloves are worn whenever there is reasonable risk of contamination of skin from spray, splatter or droplets during aerosol generating procedures. Gloves are used starting with the initial work of observing the outside of the container for external contamination.
  o Gloves are not required when observing cultures outside of the BSL-3.
- As routine work practice, the laboratorian removes all outer protective clothing when leaving the laboratory. Whether wearing gloves or not, thorough washing of hands after completion of procedures is required.

4.4.2 Respiratory protection.
• Wearing a respirator, such as N-95, is highly recommended for protecting the laboratorian when processing and manipulating specimens or TB cultures. Surgical masks should not be worn because it is designed to contain the aerosols expelled by the user not to protect from aerosols. Personnel must be fit tested prior to using an N-95. If personnel cannot be successfully fit tested for an N-95, for example an individual with facial hair, an acceptable alternative is a powered air purifying respirator (PAPR).

• No BSC is 100% effective and failures do occur; hence, respirators provide added protection.

• Personnel working in the TB Laboratory must adhere to the facility’s respiratory protection program meeting OSHA (5) requirements.
  
  o The components of the respiratory protection program are: a written standard operating procedure, training, storage of the respirator if they are to be reused, inspection of the respirator before use, medical review, and program evaluation.
  
  o Eligibility to participate in the respirator program should include a medical review and a pulmonary function test.
  
  o Before use, the worker must be fit-tested to determine the size of respirator which best fits them to ensure a tight seal to the face.
  
  o The annual fit testing is an opportunity for personnel to demonstrate proper donning of the respirator.

• The PPE requirements of personnel must also be followed by outside service technicians. The Laboratory should not permit servicing, cleaning, or
checking of the BSL-3 equipment unless a trained technical or professional person is present to ensure that adequate safety precautions are followed.

- PPE worn in the BSL-3 is removed prior to exiting the laboratory. Hands are always thoroughly washed after removal of PPE. Likewise, PPE worn in the BSL-2 must be removed prior to exiting the laboratory. Laboratory coats, used while working in the lab, are never worn outside the lab.

4.4.3 Disinfection

- A disinfectant for the TB laboratory is selected based on the tuberculocidal activity and should be categorized as intermediate activity level (1).
  - The compounds commonly selected are phenolics, iodophors, chlorine compounds or alcohols. The killing time of germicides is never instantaneous and exposure times and matrix of contaminated material must be considered when choosing an appropriate disinfectant.
- Daily disinfection of all surfaces in the TB Laboratory is required since TB is very resistant to drying and can survive for long periods on solid surfaces.
- A good disinfection practice is to soak a gauze pad or paper towel in disinfectant and place it on the work surface inside the BSC while processing specimens.
- When decanting fluids in the BSC, use a splash-proof container. Splash-proof containers need to have disinfectant added to them prior to use. If the splash-proof container has a funnel, then rinse it with disinfectant after use.
- Use a loop incinerator device or an alcohol sand flask to remove large clumps of organisms from wire loops or spades.
4.4.4 Decontamination and disposal of Laboratory waste

- An autoclave should be available in the mycobacteriology laboratory so that generated waste can be sterilized prior to transporting from the laboratory.
  - The autoclave should have proper QC and maintenance performed as scheduled.
- Chemically disinfect waste materials prior to removal from the BSC.
- If an autoclave is not available or for items that cannot be autoclave, all waste from the mycobacteriology laboratory must be securely contained in leak-proof containers. Waste should be packaged so that the outside of the container can be disinfected before it leaves the laboratory.

4.4.5 Spill clean up

- The response in the event of a spill can be categorized according to aerosols produced. The decision to follow a minimal aerosol or major aerosol spill response procedure is made in conjunction with the supervisor, the safety officer and in accordance with the biosafety manual. See Table 1 (from: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5417a1.htm)
If minimal aerosols are produced such as a spilled specimen, cover the spill with absorbent paper towels and flood with tuberculocidal disinfectant. Let the disinfectant stand on the spill until reentry.

Disinfect floors and countertops.

In the event of a major aerosol producing spill or breakage such as a liquid culture containing *M. tuberculosis*, immediately evacuate the lab. Reentry cannot occur until enough air exchanges to remove droplet nuclei from the environment as determined in the biosafety manual, typically this is around 4 hours. The supervisor or safety officer may determine it is necessary to decontaminate the lab with formaldehyde gas or other agent. Appropriate respirator protection and other PPE must be worn to clean up spills or broken material. Do not pick up broken glass with hands.

### 4.5 Clean vs dirty areas of the Laboratory

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**TABLE 1. Air changes per hour (ACH) and time required for removal efficiencies of 99% and 99.9% of airborne contaminants**

<table>
<thead>
<tr>
<th>ACH</th>
<th>99%</th>
<th>99.9%</th>
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<tbody>
<tr>
<td>2</td>
<td>138</td>
<td>207</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>104</td>
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<tr>
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<td>46</td>
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<td>3</td>
<td>6</td>
</tr>
<tr>
<td>400</td>
<td>&lt;1</td>
<td>1</td>
</tr>
</tbody>
</table>

*This table can be used to estimate the time necessary to clear the air of airborne *Mycobacterium tuberculosis* after the source patient leaves the area or when aerosol-producing procedures are complete.

† Time in minutes to reduce the airborne concentration by 99% or 99.9%.
• The laboratory work area must remain uncluttered and arranged so that the flow of material is from a clean area to a dirty area of the laboratory.

• Inside the BSC, the immediate work area is covered with a tuberculocidal disinfectant-soaked pad to capture any drops or splatter that may result from manipulation of the specimen, pipettes, loops, tubes, slides etc. If the gauze pad dries during work processes, rewet it.

4.6 AFB Blood Cultures

• Blood submitted for mycobacteria analysis should not be processed with the routine blood cultures. The specimen of choice is whole blood and should be processed in the BSL-3 laboratory.
  o If it is necessary to process as a routine blood culture for mycobacteria, all work performed on a positive blood culture should be performed in the BSL-

4.7 Instrumentation

• An aerosol proof centrifuge with a safety-shield rotor is required for specimen centrifugation which may contain live tubercule bacilli.

• Decontaminate specimen tubes and place them into domed o-ring sealed safety cups inside the BSC before transporting to the centrifuge or place the decontaminated tubes into a rack and carry to the centrifuge. After centrifugation, the unopened tubes should remain in the carrier until inside the BSC followed by decanting into a splash-proof container.
• Install a sink equipped with either an automated motion detecting faucet, knee or foot controls.

4.8 Rapid Testing (direct molecular test kits)

• Perform all work within the BSL-3 laboratory and within the BSC.

• Once the sample on which a rapid test will be performed has been inactivated or genetic material extracted, further testing can be performed in a BSL-2 laboratory setting.

4.9 Molecular Testing

• All work involving processing specimens suspected of containing tubercle bacilli and manipulation of mycobacterial cultures must be performed within the BSL-3 Laboratory and within the BSC.

• Once the sample on which a molecular test will be performed has been inactivated or genetic material extracted, further testing can be performed in a BSL-2 Laboratory setting.

References:


5  Necropsy/Autopsy; Surgical Pathology

5.1  Autopsy associated infections
    5.1.1  Blood borne infections
    5.1.2  Other infections
    5.1.3  Infectious aerosols
    5.1.4  Organisms that require additional safety practices
    5.1.5  Other biosafety exposures
    5.1.6  Reporting to the funeral home

5.2  The Autopsy suite
    5.2.1  Inspect the body
    5.2.2  Guidelines for the suite

5.3  Chemicals – formaldehyde

5.4  Spills

5.5  Personal protective equipment
    5.5.1  Personal precautions
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5.6  Waste management
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5.0 Necropsy/Autopsy; Surgical Pathology

- **Autopsy (human cadaver examination)** - The infectious and hazardous risks to the laboratory worker performing an autopsy are higher than that for any other healthcare professionals because of the procedures used, population being assessed, and performance of work in an open area. Use of scalpels, saws, and needles as well as the exposure to sharp objects within the body, bone fragments, fractured metal and/or needles, can result in cuts and percutaneous injuries. Manipulation of large organs creating body fluid and blood splashes, and use of instrumentation, such as hoses and saws, creates aerosols in an open area that can result in inhalation, direct contact or contact of contaminated items in the environment. Those involved in the autopsy directly as well as those in the room are at risk for exposures. Other potential safety risks include exposure to chemicals, such as formalin, therapeutic radiation beads, and retained electrical hardware. An alert and well-trained worker, facility design, optimally fitting and user friendly PPE, appropriate surgical procedures during manipulation of the body and dissected material, as well as subsequent disinfection and sterilization procedures are critical in minimizing biosafety risk during autopsy and embalming.

- **Necropsy (animal cadaver examination)** - The risk of laboratory acquired infection is very different when working with human cadavers (where infectious agents in essentially all the cadavers are infectious to humans) compared to animal cadavers (where infectious agents in most cadavers are not human pathogens). Regardless, animal cadavers can harbor zoonotic agents and risk assessment to determined whether or not zoonotic infectious agents may be present in a cadaver, as outlined in section 12, is critically
important for establishing appropriate animal necropsy biosafety procedures. The guidelines below are combined biosafety best practices for both human autopsy and human surgical pathology, and animal necropsy and veterinary surgical pathology. When necessary, biosafety guidelines distinct for human or animal diagnostic laboratory settings are highlighted.

5.1. **Autopsy/necropsy associated infections.** The cause of most laboratory acquired infections and hazardous exposures that occur during autopsy/necropsy are unknown and all autopsies and necropsies should be considered risky (1,2).

- Human autopsy facilities should preferably be able to function at a Biosafety Level 3 for optimal protection of those involved directly with the autopsy and personnel in the surrounding area (1,2,3,4).

- Animal necropsy facilities should be able to function at a Biosafety Level 2 with an option for Biosafety Level 3 practices when warranted from “case by case” risk assessment (e.g. availability of Class II Biosafety Cabinet, down draft necropsy tables, appropriate personal protective equipment such as eye and face protection). Only if risk assessment indicates a high probability for the presence of a high consequence livestock pathogen (USDA Livestock Select Agent or Toxins-See section 12, Table 4) would Biosafety Level 3 facilities be required.

- The Medical Director is responsible for risk assessment and for consideration of limited autopsy/necropsy procedures and subsequent acceptable risk level to personnel prior to each autopsy/necropsy (1, 2, 5, 6) or in the case of animal necropsy, the attending pathologist.
A staff member should be trained in safety procedures and given oversight of safety procedures and risk analysis in the Pathology suites.

5.1.1. **Blood-borne pathogens.** Human-healthcare workers involved in performance of autopsies are a high-risk group for occupationally acquired blood-borne pathogens due to both the injuries sustained as well as the population undergoing autopsy. Transmission risk is highest per exposure for Hepatitis B Virus> Hepatitis C Virus> HIV, respectfully. These infections have been documented from autopsies as well as during embalming (1,2,5,7-9).

5.1.2. **Other infections.** Specific data for other blood-borne pathogens such as Cytomegalovirus are lacking, but infectious transmission is possible and risk may be higher especially for pregnant (serologically negative) or immunocompromised workers. Individuals at higher risk for infection should be assessed on a case by case basis and consent to participating in the autopsy after being counseled (5,10).

5.1.3. **Infectious aerosols.** Autopsies/necropsies from suspect zoonotic agent cadavers generate potentially infectious aerosols. While *Mycobacterium tuberculosis* is the prototypical pathogen most noted to be transmitted by aerosolization, patients with meningococcemia, anthrax, rickettsiosis and legionellosis are other examples. Manipulation of infectious tissue can result in both airborne particles in a size (< 5 um) that floats on air currents for extended periods of time and can subsequently reach the pulmonary alveoli as well as small-droplet particles (> 5 um) that settle more quickly. Contamination may occur from fluid aspirating hoses, spraying the body, and oscillating saws. The aerosols created stay within the autopsy area and can result in subsequent contact
with mouth and eyes, inhalation or ingestion, as well as contaminate inanimate surfaces such as computers, phones and camera equipment (2,3).

5.1.4 Organisms that require additional safety practices

- No cases of autopsy acquired Creutzfeldt-Jakob disease (CJD) have been documented. However, because the prion infectious particle cannot be rendered non-infectious by normal decontamination and sterilization methods, enhanced precautions are mandatory. Transmissibility of the prion is retained in formalin-fixed paraffin blocks (11-14).

- The only natural animal prion disease with known zoonotic infection potential is “classical” bovine spongiform encephalopathy (BSE). Necropsy guidelines for cattle with suspected BSE are published elsewhere and focus primarily avoiding skin puncture, reducing splashes onto mucous membranes, appropriate decontamination of facilities and equipment, and appropriate carcass disposal (Biosafety Guidelines developed for National TSE Veterinary Diagnostic Laboratory Network. Canadian Food Inspection Agency. Version 1.0, March 8, 2002).

5.1.5 Other biosafety exposures

- Cyanide, metallic phosphides and organophosphate pesticide poisoning.
• Specific precautions are required and may include use of a fume hood or Class II Type B2 BSC that is ducted to the outside, fume respirators, limiting the autopsy/necropsy, and limiting the time of exposure (2).

• Diagnostic radioactive beads or therapeutic scans.
  ○ Consult the radiation safety officer of record for recommended appropriate measures to limit the exposure of radiation, transfer of the body, and post-exposure testing of personnel.

• Electrical and other hardware hazards (pacemakers, indwelling catheters).
  ○ Deactivation of pacemakers should be undertaken before autopsy continues. Discharge of electrical current is possible when defibrilators are present.
  ○ Clamp or remove indwelling hardware before the body is transported to the autopsy/necropsy suite. If hardware exists, it should be noted and then removed so as not to cause cutting injuries during the autopsy.

5.1.6 Reporting to the funeral home. Report known blood-borne pathogen or other suspected aerosolization danger to the funeral home and others potentially handling the body to limit subsequent transmissions that may occur during transport or embalming (15).
5.1.7 **Necropsy remains of animals.** Cadavers with potential zoonotic infectious agents should be disposed of by appropriate decontamination (e.g. incineration, alkaline digestion or other methods), and not be returned to animal owners for private burial.

5.2 The Autopsy/Necropsy Suite

5.2.1 **Inspect the body/carcass.** Search for implanted items retained after death. These should be noted and clamped or covered prior to transport to reduce body fluids oozing from the body. The body should be cleaned of visible bloody/body fluids. Cover the autopsy table with a plastic sheet to retain the majority of fluids or tables should contain drains so that fluids may be collected in buckets or floor drains.

- Fluids and tissues from necropsy of animals with suspected zoonotic agents should be appropriately disposed of by methods that provide adequate decontamination, depending upon the specific suspected infectious agent (e.g. incineration, rendering, composting).

5.2.2 **Safety guidelines for the suite.**

- Use the universal biohazard symbol to mark the autopsy suite as a biohazards area at the entrance.

- Secure access to the autopsy suite and grant access only to those personnel trained in the biosafety procedures specific to this area.

- Protect vacuum hoses with liquid disinfectant traps and HEPA filters or their equivalent.

- Use hand saws whenever possible to reduce aerosols. Moisten bone before cutting. Fit oscillating bone saws with a vacuum attachment and use them in a closed area if possible.
When cutting the skull during autopsy, the head should be bagged. Any subsequent jagged edges of exposed bone should be covered with towels.

- Do not leave used needles on the table. Do not detatch or resheath needles. Discard the whole unit into sharps containers. Make sure that sharps containers are available in this work area, and inspect them periodically to ensure that they are never more than 2/3 full. Seal off and replace them when they reach this level.

- Limit the number of personnel working on the human body at any given time to the prosector and/or physician and circulator. Only one person should be cutting at a given time. The same number limitation could apply to small animal necropsy. Large animal necropsy generally requires multiple prosectors working together in a way to avoid accidental lacerations.

- Prepare multiple scalpels prior to autopsy so blade changes while hands are slippery and contaminated can be avoided. Use blunt-ended scissors when possible instead of scalpels and use a magnet to pick items from the table if slippery.

- Do not pass sharp objects such as scalpels or scissors to another person. Placed them on the table for another person to pick up.

- Place specimen containers (e.g. blood culture bottles) on the table for inoculation. Use a rack if possible. Do not hold in the hands while inoculating.

- Examination of organs in the body and evisceration technique should be considered to limit exposure to blood, body fluids and cuts.

- For unfixed tissue that will be removed from the autopsy table:
  - Place on a tray or in a bucket to avoid splashing.
○ Post examination, cutting and/or photography tissue should be returned to the autopsy table to be replaced in the body and/or fixed.

○ Specimens that will be submitted for culture or other laboratory tests should be placed in a primary container which is surface decontaminated on the outside and then placed into a secondary leakproof container and labeled as biohazard.

○ Large organs will have to be removed and cut into multiple sections (breadloved) so that adequate permeation of the tissue for fixation will occur.

○ Unfixed tissue that will not be returned to the body is considered biohazard waste and should be kept to a minimum, and subsequently disposed of in a manner to allow appropriate decontamination.

○ For autopsy, either suture or staple the body closed. Hold skin flaps with forceps, not hands, when suturing.

○ Review of any unfixed tissue requires the use of the same PPE as that used in the autopsy.

● Use hands-free or foot activated recording devices during dictation and hands free speaker phone to minimize contamination of inanimate surfaces.

● Provide a hands free sink at the exit for washing.

● Eye wash station and shower should be available (5).
Formaldehyde (3.7 - 4.0%), used for specimen preservation is the most common toxic chemical to which autopsy workers are exposed. The chemical is volatile and toxic and causes irritation to the eyes, mucous membranes and skin and is associated with increased risk for all cancers. The Occupational Safety and Health Administration (OSHA) has regulated exposure limit as 0.75 ppm as an 8-hour time-weighted average and to 2.0 ppm for short-term (15 minute) exposures (16). If formaldehyde can be detected by smell it likely means exposure is occurring at a concentration beyond acceptable limits.

Limiting exposure:

- Cover all specimen buckets where organs may be deposited for fixation.
- Collect discarded formalin soaked towels and other formalin soaked waste in a bag at the grossing table. Periodically spray a formalin neutralizing agent on the waste as it is filled. Seal off the bag when it is filled.
- Discard bagged formalin soaked towels and other waste in a lined container that can be opened and closed with a foot pedal.
- Cut large fixed organs in a fume hood or downdraft table.
- Monitor workers and resident pathologists with formaldehyde monitoring badges for 8 hour periods and at least 15 minute periods periodically to assess formaldehyde exposure.
- Ensure that tissue grossers are competent in proper tissue grossing technique.

5.4 Spills.
Use neutralizing, absorbent mats for small spills. Neutralizing reagents provide a convenient, cost-effective method for the disposal of hazardous formaldehyde, gluteraldehyde, and other aldehyde solutions. They convert hazardous aldehydes into a nonhazardous, noncorrosive, nontoxic polymer and water. The polymer produced is not a hazardous waste (as defined by United States Title 40 Code of Federal Regulations (40 CFR 261.24(a)). These neutralizing agents tend to reduce disposal costs and contribute to a safer work environment. In some cases after formaldehyde waste treatment with crystal products, the resulting solid waste may be discarded in approved laboratory solid waste streams.

Wear appropriate protective gloves and protective clothing to prevent skin exposure. Wear protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133. Do not wear contact lenses when working with formalin.

5.5 Protective equipment

5.5.1 Safety equipment

Biosafety Cabinets (2,5). Biosafety cabinets are not common in autopsy suites because of limitations due to the volume and size of material being manipulated.

Necropsy facilities that have Class II biosafety cabinets should use them when practical (for small animals) for necropsies of cadavers with
suspected zoonotic agents as indicated by a “case by case” risk analysis. However, large animal necropsy of cadavers with suspected zoonotic agents is not practical in biosafety cabinets. Thus PPE, engineering controls and procedures are used that have been clearly developed for clinical laboratories.

● For optimal protection when there is a risk of exposure with agents from blood-borne pathogens and from agents transmitted by aerosols, all autopsy/necropsy facilities should be able to use Biosafety level 3 work practices and physical containment, or reference the work to a facility that does (2, 6).

● Airflow should be unidirectional from clean areas to dirty areas.

● The room should be under negative pressure relative to other surrounding rooms, with 12 air exchanges per hour.

● Air can be directly vented to the outside or re-circulated into the room via HEPA filters but should not be allowed to re-circulate into any clean surrounding areas as this has been associated with outbreaks of *M. tuberculosis*.

● In the event of redesign of the autopsy/necropsy suite, placement of a BSC should be considered to mitigate exposure to infectious tissues (2, 17).

**5.5.2 PPE for autopsy/necropsy personnel**

● Clothing.
○ Fluid-resistant (surgical) jumpsuit or shirt and pants that cover from neck to feet and arms.

○ Water-proof apron and water-proof sleeves.

○ Closed-toe shoes covered with fluid-proof shoe covers or booties.

○ Surgical cap or hood bonnet that covers head entirely (autopsy only).

● Protection from splashes and aerosols.

○ For general autopsy, or for necropsy when a risk assessment indicates a high likelihood for zoonotic agents in the cadaver and the necropsy cannot be conducted in a Biosafety Cabinet, use a transparent face-shield that covers eyes, mouth and neck and a fit-tested N95 respirator. Eye glasses and contacts alone are not adequate for protection.

○ Persons unable to wear N95 respirators should wear powered air-purifying respirators (PAPRs).

○ Surgical masks do not protect autopsy participants from inhaling airborne contaminants such as infectious respirable aerosols or hazardous chemical vapors (2,3,15,17,19).

● Gloves.

○ Double-gloving is recommended throughout the autopsy/necropsy and gloves should be changed every hour. 8% of
gloves are punctured during autopsy and about 1/3 of punctures remain undetected until after de-gloving (2, 18).

- Select for autopsy, a cut-resistant glove of fine-woven steel to prevent cuts from bone and scalpels, and subsequently covered with a rubber glove for slip-resistance. Cut-resistant gloves do not protect from needle sticks.

- Select gloves specific to the particular task based on the case. For example, heavy gloves for chemical exposure, or gloves that resist accidental puncture if needles will be used.

- Gloves should be changed immediately upon recognized puncture and hands washed with soap and water before resuming the autopsy/necropsy.

- Cover cuts or abrasions with water-proof occlusive bandages for everyone participating in the autopsy.

- Provide PPE for personnel present but not directly participating in the autopsy, i.e. medical students/observers. The same PPE should be used for observers as those participating in the autopsy/necropsy.

- All surfaces are expected to be contaminated.

- Human autopsy: Disinfection and Cleaning Procedures for Equipment, Instruments

  - Clean any spills of body fluids or tissues immediately. Cover the spill with paper towels and saturate the area with a disinfectant or a
detergent solution; or use a mop followed by disinfection of the spill area with 10% bleach.

○ Decontamination of the body post autopsy. Wash the body with a detergent solution, followed by an antiseptic solution or diluted bleach. Place the body in a leak-proof shroud and label as biohazard. Direct notification of the mortician is appropriate in cases of known blood borne pathogens or the presence of *M. tuberculosis*.

○ Work surfaces. Wash all work surfaces and floors with a detergent solution followed by disinfection with 10% bleach solution.

○ Instruments. Remove visibly gross tissue from non-disposable items (knife handles, pans, aprons) before chemical disinfection or autoclaving. Place sharp objects in puncture resistant containers and wash with detergent solution to remove any remaining gross tissue, rinse with water and wet thoroughly with 10% bleach solution depending on amount of blood and body fluid present. 2% aqueous gluteraldehyde is preferred for stainless steel and aluminum devices since bleach will corrode these items. Remove the gluteraldehyde or bleach by rinsing with water.

○ Place disposable items, paper products, aprons, sponges, etc into a biohazard container or bag for terminal treatment or autoclaving.
○ Place reusable clothing in a leak proof biohazard bag for laundering according to hospital protocol.

○ Wash reusable, non-launderable items such as aprons with a detergent solution, decontaminate with bleach solution, rinse with water and allow to dry before next use.

○ Camera, phones, computer keyboards, etc. should be kept as clean as possible but should be considered to be contaminated and handled with gloves. The items should be wiped clean with detergent solution and 10% bleach solution after each use.

○ Place reusable clothing in a leak proof biohazard bag for laundering according to hospital protocol.

○ Wash hands upon removal of gloves using soap and water when exiting from the room.

● Animal necropsy room disinfection and cleaning procedures for equipment and instruments

○ Anyone involved in the body, room or instrument clean up should wear the appropriate PPE.

○ Work surfaces. Decontaminate all work surfaces and floors with a detergent solution followed by disinfection with a 10% bleach solution.

○ Instruments. Remove visibly gross tissue from non-disposable items (knife handles, pans, aprons) before chemical disinfection or
autoclaving. Place sharp objects in puncture resistant containers and wash them with detergent solution to remove any remaining gross tissue, rinse with water and wet thoroughly with 10% bleach solution depending on amount of blood and body fluid present.

○ Put disposable items, paper products, aprons, sponges, etc in a biohazard container or bag for terminal treatment or autoclaved.

○ Place reusable clothing in leak proof biohazard bag for laundering according to hospital protocol

○ Wash reusable non-launderable items such as aprons with a detergent solution, decontaminate with bleach solution, rinsed with water and dry before next use.

○ Keep camera, phones, computer keyboards, etc. as clean as possible but consider them to be contaminated and handle them with gloves. Wipe the items clean with detergent solution and 10% bleach solution after each use.

○ No dirty items or anything used in the autopsy is removed from the room, including clothing and laboratory coats.

○ Wash hands upon removal of gloves, with soap and water upon existing from the room

5.6 Waste management

5.6.1 Tissue.
• Human tissue: Incinerate all pathological waste since this is considered hazardous material and is regulated by the U.S. DOT or transport pathological waste to on-site or off-site treatment facilities in clearly labeled, dedicated, leak proof containers or carts that meet the U.S. DOT requirements. U.S. DOT Sharps waste containers need to be puncture proof in addition to the above requirements. State, local, and regional regulation may also apply and need to be addressed.

• Animal tissue: Dispose of all animal necropsy waste (tissues or post-necropsy cadaver) using a method appropriate for the “case by case” risk analysis assessment (incineration, autoclaving and standard waste disposal, rendering, composting, cremation, private burial).

5.6.1 Other waste.

Autoclave red-bag waste. Shred if appropriate. State, local, and regional regulation may also apply and need to be addressed. See section 3.5.

5.7 Clean versus dirty areas

Clean areas may include an administrative area and bathrooms with showers. Air from these areas should be exhausted differently than the autopsy suite (2, 20, 21). All other areas are considered dirty and appropriate PPE is required.

5.8 Surgical Pathology
● Surgical pathology includes gross dissection and frozen sectioning. Histology and cytology are hybrid areas of autopsy and the clinical diagnostic laboratories. Each laboratory section has its own unique safety issues. Very limited data exists on standardized biosafety practice as well as reported biosafety incidents.

● Documentation and research in surgical pathology safety are necessary for future guidelines and recommendations (22,23). Surgical pathology risks are associated with manipulating large amounts of fresh tissues from unknown infectious sources that may result in puncture, cuts and splashes of blood and body fluids similar to the autopsy where fresh organs must be viewed at a grossing table and “breadloaved” or cut into sections thin enough for fixation. Other risks include the use of cryostat cutting equipment or freezing spray that generate infectious aerosols when sectioning frozen tissue, and exposures to large volumes of formaldehyde.

● Tissue that will be used for slide examination is fixed in formalin, and subsequently small tissue sections of interest are dissected with scalpels, placed in cassettes, replaced in specimen vats with formalin and transported to histology.

● Histology takes formalin-fixed tissue and then embeds the tissue in paraffin, cuts these sections with microtome-bladed instruments to make slides for viewing using specific stains and immunochemistry. While most formalin-fixed specimens are non-infectious, both Mycobacterium tuberculosis and CJD have been transmitted to histology technicians upon cutting of formalin-fixed sections. Exposure to toxic formaldehyde is common.

● Cytology receives large and small volume body fluids, bone marrow samples or needle aspirate specimens most of which are received in fixative but others that must
be processed by aliquotting or pouring off large volume body fluids can result in splashing and spills. Procedures such as centrifugation and cytospin processing can produce aerosols. Air-dried slides can be a source of contamination until fixed and stained.

5.8.1 Specimen receiving and Log-In

- Handle specimens with Standard Precautions and wear gloves when receiving and accessioning (3,5). Receipt areas in all surgical pathology laboratories should be considered dirty areas and all exterior containers considered contaminated.

- Submit specimens delivered from the operating room, autopsy, or from outside collection sites in leak-proof containers or place in fixative specimen containers at the site of collection (i.e. skin or gastrointestinal biopsies). Place the specimen in a secondary leak-proof bag or container and label as a biohazard with the requisition in an outside pocket to avoid contamination with the specimen.

- Leaking specimens or visibly contaminated specimens.

  Specimens may be contaminated with fresh tissue, blood, or formalin. Handling is based on whether the specimen is fresh or formalin-fixed.

  - If the specimen is submitted in fixative and the secondary container is not leaking, tighten the specimen cap and place the primary container in a clean bag and wipe any formalin from the bench top.

  - If the specimen is submitted as fresh tissue and the exterior container is leaking, place the specimen into another container and process it using
someone with appropriate PPE at which time specimens should be transferred to a clean container and labeled.

○ If the requisition is contaminated, discard it as biohazardous waste and replace it.

5.8.2 Work at the Open Bench

● Surgical pathology grossing station set-up

○ Separate the log-in room and administrative areas from the grossing room if fresh tissue or cryostats are used in the same open area (2, 20, 21).

● Handling of fresh tissue

○ Examine fresh tissue in a BSC if possible, or in a room separated from the rest of the surgical pathology grossing stations, using PPE similar to autopsy conditions depending on volume of blood and body fluid exposure likely (2). Fresh tissue should never be handled without gloves.

○ For human pathology, store fresh tissue not undergoing fixation or unable to be adequately fixed, such as teeth or foreign bodies in a double, sealable, leak proof container and label as biohazard and store in a refrigerator or freezer (5).

○ Risk reduction may be aided by obtaining patient history prior to collection of tissue and/or performance of fresh frozen sections (23).

● Frozen sections

○ Frozen sectioning is a high risk procedure for infectious exposure and is performed on fresh tissue. Freezing tissue does not kill organisms and the use
of the cryostat cutting blade creates potentially dangerous aerosols. The true clinical necessity for frozen-sectioning should be discussed with the surgical team.

○ Although some cryostat instruments have a downdraft into the instrument, aerosols are dispersed into the room where the cutting takes place. Likewise, freezing propellant sprays which speed the freezing process by a few seconds, causes aerosolization of not only the tissue being frozen but also the tissues from previously cut specimens that are at the base of the instrument. Thus, both procedures generate aerosol and droplet contamination generating an infectious risk to all personnel in the area (2, 23, 26). Therefore, Do not use freezing sprays.

○ Some cryostats have ultraviolet lights but these are not a substitute for terminal cleaning of the instrument and have been shown to be ineffective in killing mycobacteria.

○ Discontinuation of freezing sprays has been recommended in other guidelines as not recommended by the manufacturers of cryostat instrumentation (5, 23). Ideally, cryostats should be used in a closed room that has air vented directly to the outside or re-circulated via a HEPA filter to avoid contamination to the rest of the surgical pathology.

○ In human pathology laboratories, gloves, face-shield or goggles, and N95 mask should be worn when processing.

● Bone cutting. See Autopsy section 5.2.2
● Fixed Tissue: Surgical pathology

○ Fix tissue in 10% formalin in a concentration 10 times the volume of tissue to ensure effective fixation and to reduce potential infectious contamination. However, fixed tissue has been shown to remain infectious and viability of infectious organisms is dependent on a host of variables that have not been clearly identified (22). *M. tuberculosis* has been transmitted from fixed specimens as well as grown from fixed specimens and CJD is not inhibited by the routine concentration of formalin. Grossing stations where formalin fixed specimens are cut are designed for decreasing the fumes of formaldehyde but are not BSCs (3,5,27-29). Formalin-fixed specimens should be handled with gloves.

● Use appropriate PPE, particularly if there is a high index of suspicion of infectious aerosol production at the grossing station (unfixed tissue present or indicated by risk analysis). Face shields are optimal for full face and neck protection if splashing with formalin is likely and offer the most comfort for extended periods of time while allowing both prescription eyeglasses and dictation.

  ○ Eye glasses and contacts are not a substitute for eye protection.

  ○ Goggles and fluid-resistant mask are an alternate choice but not preferred.

  ○ Face shields/goggles should be able to be decontaminated or disposable.

● Keep containers with tissues in formalin closed for reduced exposure fumes.
● Cytology specimens. PPE is dependent on the specimen. Gloves and lab coat are required for all specimens until slides are fixed and stained.

○ Pour off or aliquot large volume specimens with potential for splashing and/or aerosolization inside a BSC and wear fluid-resistant clothing and apron and two pairs of gloves.

○ Open small volume body fluids or aspirates submitted in tubes in a BSC or use a splash guard or a face shield and aliquot by disposable pipette rather than pouring to avoid splashing and spill.

○ Handle specimens received in fixative with gloves due to toxicity of the fixatives and external contamination (3). If slide preparation will use a cytospin preparation, the system should include a bowl with safety lid and outside cover.

○ Consider all slides, impression smears, cytological preparations, bone marrow smears, as infectious until fixed and stained.

○ Use a safety centrifuge with safety cups with O-rings and sealable tops for centrifugation of fluids (30).

● Decontamination

○ Cryostat - instrument shavings generated by cutting are considered contaminated. Accumulated cuttings should be collected and discarded as biohazardous waste. Defrost the instrument and decontaminate daily with 70% alcohol. Decontamination should occur weekly with a tuberculocidal
disinfectant or after a known case of *M. tuberculosis*. Wear stainless steel mesh gloves while cleaning the microtome knives.

- Microtome – consider a similar schedule as with cryostats.

- Consider all surfaces, computer, phone, counters as contaminated if any persons using gloves touches these items. Disinfect equipment and bench tops daily.

- Remove gloves and wash hands with soap and water before exiting the various laboratory rooms.

### 5.8.3 Clean vs. dirty areas of the laboratory.

- All of the surgical pathology specialty areas (cytology, histology, grossing or frozen section rooms) should be considered dirty areas if fresh specimens or body fluids are received or processed in an open room (not in a BSC or separately vented area).

### 5.8.4 Tissue stains

Multiple staining procedures exist in histology and cytology. The most common are included here. Some of these stains are prepared with ethanol and some with methanol that can significantly impact management options for their waste. MSDS sheets for each component should be available in the laboratory.

- Hematoxylin Stain
Hematoxylin stain is not hazardous under EPA regulations. Drain disposal is recommended with the permission of local wastewater treatment authorities. Follow federal, state and local regulations.

• Giemsa Stain

The preferred disposal method is incineration via a permitted hazardous waste treatment facility. Localities may restrict the amounts of alcohols that may be flushed down the drain and should be consulted. Insure compliance with all local, state, and federal government regulations.

• Wright Stain

Wright stain contains methanol, a listed hazardous waste. Sewer disposal of listed hazardous wastes is not acceptable or permitted. The preferred disposal method is incineration. Insure compliance with all government regulations.

5.8.5 Fixatives

• Formalin (HCHO)

Formaldehyde may be purchased as a 37 to 40% HCHO solution; however, for dilution, it should be considered to be 100%. Diluting these concentrations of formalin is discouraged to limit exposure. The most common formalin preparation is 10% formalin which is available commercially. Incineration is the preferred disposal method for formaldehyde. Local governments often restrict the amounts of aldehydes that may be flushed down the drain. Each laboratory must comply with all government regulations.
Neutralizing reagents provide a convenient, cost-effective method for the disposal of hazardous formaldehyde, glutaraldehyde, and other aldehyde solutions. They convert hazardous aldehydes into a nonhazardous, noncorrosive, nontoxic polymer and water. The polymer produced is not a hazardous waste (as defined by United States Title 40 Code of Federal Regulations (40 CFR 261.24(a)). These neutralizing agents tend to reduce disposal costs and contribute to a safer work environment. In some cases after formaldehyde waste treatment with crystal products, the resulting solid waste may be discarded in approved laboratory solid waste streams. Before engaging in sewer disposal of neutralized formalin solutions, be sure to have formal approval of applicable local wastewater authorities.

Wear appropriate protective gloves and protective clothing to prevent skin exposure. Protective eyeglasses or chemical safety goggles should be worn as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166. Contact lenses should not be worn when working with formalin. Maintain eye wash fountain and drench facilities in work area.

Other pathology fixatives

Fixatives are classified as regulated waste. OSHA’s Blood borne Standard 29 CFR Part 1910, 1048, and 1030 mandates that pathology specimens be placed in secondary containers that are constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping.

Labeled as biohazard
● Xylenes and alcohols

○ Xylene is categorized as a hazardous waste under RCRA and has been assigned EPA Hazardous Waste No. U239. Waste xylene can be separated from dissolved paraffin by distillation, and there are a number of commercially available recycling units that can accomplish this separation effectively to produce reusable xylene. Xylene also may be disposed of through a properly permitted hazardous waste contractor using an organic metallic or organic laboratory pack that meets the requirements of 40 CFR 264.316 or 265.316.

○ Xylene that cannot be saved for recovery or recycling must be handled as hazardous waste and sent to a RCRA approved incinerator or disposed in a RCRA approved waste treatment facility. Processing, use, or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

○ The disposal guidelines for alcohols (ethyl alcohol, isopropyl alcohol, methyl alcohol) are identical to those for xylene indicated above.

● Testing for formalin and xylene exposure

○ Conduct periodic testing with chemical badges for an 8 hour period and at least one 15 minute period periodically to assess formaldehyde exposure in individuals
that are routinely exposed (technologists, tissue grosser, resident pathologists, etc) and rotated among various areas so that every work station is assessed.

5.9 Engineering controls and facility renovations.

● Updates and renovations to autopsy and other areas of anatomic pathology that process fresh tissue and body fluids should consider:
  ○ Designing closed specimen receiving/administrative areas for receiving specimens if BSC are not available for processing fresh specimens or if cryostats are in the same area as administrative personnel.
  ○ Providing rooms with negative air flow relative to surrounding rooms.
  ○ Installing a BSC for processing fresh tissues.
  ○ Providing a separate room for cryostats and bone saws relative to the remainder of the surgical grossing suite.

5.10 Creutzfeld-Jakob Disease (CJD)

Special autopsy precautions and autopsy suite decontamination, brain cutting and histologic tissue preparation procedures are required when processing cases of possible CJD. Detailed steps for precautions for autopsies of subjects with suspected prion disease, autopsy suite decontamination procedures, brain cutting procedures, and tissue preparation are presented in reference 2, tables 4-7 and in other sources (1, 12, 14, 31).
5.10.1 Autopsy. Autopsies should be performed using BSL-2 precautions augmented by
BSL-3 facility ventilation and respiratory precautions wearing standard autopsy PPE.
The autopsy should be limited to brain removal and only absolute necessary persons
required should be present. The brain should be double-bagged and placed in a plastic
container for freezing or fixed in 3.7% to 4% formaldehyde after sectioning.
Formaldehyde fixation occurs for 10-14 days before histological sections are collected.

5.10.2 Histologic preparations: Prions are not inactivated by formalin and transmission
has occurred from paraffin-embedded blocks handled in histology (2,11,13,31).

- PPE includes, gloves, lab coat, apron, and face protection.

- Every component of histological preparation is processed by hand with
disposable items.

- Before histologic slide preparation, small blocks of tissue from the brain ≤ 5 mm
thick are soaked in 95% to 100% formic acid for 30 minutes to one hour, followed
by soaking in fresh 4% formaldehyde for at least 48 hours.

- Tissue is embedded in disposable embedding mold and then embedded in
paraffin and sections prepared.

- All waste is collected and the knife used is discarded as bioharzed sharps and
waste.

- Slides are labeled as Infectious CJD and handled with “CJD precautions”. The
sectioned block is sealed with paraffin. Slides are processed by hand with
reagents prepared in disposable specimen cups or Petri dishes.
● After placing a coverslip, slides are decontaminated by soaking for 1 h in 1-2 N NaOH.

● Equipment is decontaminated as above (collected and disposed as biohazard waste for incineration) and equipment, tissue remnants or contaminated formaldehyde solution is discarded as infectious hospital waste for incineration.

● Dedicated equipment may be required depending on local jurisdictions.
References:


10. CDC, FDA, NIH. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis


6. Parasitology laboratory

6.1. Specimen receiving and log-in/set-up station
   6.1.1. Leaking containers
   6.1.2. Visual contamination on outside of container
   6.1.3. Loose caps

6.2. Stains and reagents
   6.2.1. Trichrome stain
   6.2.2. Hematoxylin stain
   6.2.3. Iodine
   6.2.4. Acid-fast stains
   6.2.5. Giemsa stain
   6.2.6. Wright stain
   6.2.7. Formalin
   6.2.8. Mercury-based fixatives
   6.2.9. Zinc-based fixatives
   6.2.10. Copper-based fixatives
   6.2.11. Xylenes and alcohols

6.3. Working at the bench

6.4. Personal precautions
   6.4.1. Biosafety cabinet vs fume hood
   6.4.2. Personal protective equipment
   6.4.3. Immunization
   6.4.4. Disinfection
   6.4.5. Disposal of laboratory waste

6.5. Clean vs dirty areas of the laboratory

6.6. Instrumentation

6.7. Antibody and antigen parasitology testing
6. PARASITOLOGY LABORATORY

Exposure to infectious parasites during diagnostic procedures may result from the handling of specimens, drawing of blood, various types of concentration procedures, organism cultures, and animal inoculation studies. Relevant parasites and their possible routes of infection are presented in Tables 6.1 and 6.2. Information on resistance to antiseptics and disinfectants can be found in Table 6.3.

6.1. Specimen Receiving and Log-In/Set-Up Station

● Fresh specimens (feces, other gastrointestinal tract specimens, urine, blood, tissues, cerebrospinal fluid, other body fluids, arthropods) represent a potential source of infectious parasites.

● Safety precautions should include: proper labeling of fixatives; designating specific areas for specimen handling (biological safety cabinets may be necessary under certain circumstances); proper containers for centrifugation; acceptable discard policies; appropriate policies for no eating, drinking, or smoking, etc., within the working areas; and, if applicable, correct techniques for organism culture and/or animal inoculation.

● Collect or transfer every specimens into a leak proof primary container with a secure lid (avoid snap-top closure).

● Use disposable plastic bags with separate pockets for the requisition slip and specimen when possible (1,2).

6.1.1. Leaking Containers

● Visually inspect all specimen containers for leakage.
● Contaminated primary containers should be decontaminated prior to further manipulation. In some circumstances, the contents could be transferred to a clean container or specimen recollected prior to submission to the testing area.

● Discard contaminated requisitions as biohazardous waste and replace them.

6.1.2. Loose Caps

● Blood specimens are submitted in tubes, usually vacutainer tubes (either lavender or green tops). If the stopper appears to be loose or there is evidence of blood on the outside of the tube, the stopper should be pushed into the tube for a secure fit and the tube should be decontaminated on the outside prior to specimen processing.

6.2. Stains and reagents

6.2.1. Trichrome Stain (16)

● Wheatley’s modification of the Gomori tissue trichrome stain is considered non-hazardous waste and must be disposed of in accordance with federal, state and local environmental control regulations.

● Drain disposal is recommended with the permission of local wastewater treatment authorities.

6.2.2. Hematoxylin Stain (4)

● Hematoxylin stain is not hazardous under EPA regulations.

● Drain disposal is recommended with the permission of local wastewater treatment authorities. Canadian disposal regulations generally parallel those in the United States.
Follow federal, state and local regulations.

6.2.3. Iodine (4)

- All liquid and iodine-contaminated material should be disposed of in Department of Transportation (DOT) approved waste containers.
- Incineration for liquids is the suggested method of disposal. Comply with all Federal, State and Local regulations for disposal.

6.2.4. Acid-Fast Stains (Modified) (4)

- Dispose of container and unused contents in accordance with applicable federal, state and local requirements.
- State and local disposal regulation may differ from federal disposal regulations.

6.2.5 Giemsa Stain (4)

- The preferred disposal method is incineration via a permitted hazardous waste treatment facility.
- Localities may restrict the amounts of alcohols that may be flushed down the drain. Insure compliance with all government regulations.

6.2.6. Wright Stain (4)

- The preferred disposal method is incineration in an approved facility.
- Localities may restrict the amounts of alcohols that may be flushed down the drain. Insure compliance with all applicable government regulations.

6.2.7. Formalin (HCHO)
• Formaldehyde is normally purchased as a 37 to 40% HCHO solution; however, for dilution, it should be considered to be 100%. Two concentrations are commonly used: 5%, which is recommended for preservation of protozoan cysts, and 10%, which is recommended for helminth eggs and larvae. Although 5% is often recommended for all-purpose use, most commercial manufacturers provide 10%, which is more likely to kill all helminth eggs. The most common formalin preparation is 10% formalin.

• Incineration is the preferred disposal method for formaldehyde.

• Local governments often restrict the amounts of aldehydes that may be flushed down the drain. Each laboratory will need to comply with all government regulations.

• Use neutralizing reagents to dispose of hazardous formaldehyde, glutaraldehyde, and other aldehyde solutions. They convert hazardous aldehydes into a nonhazardous, noncorrosive, nontoxic polymer and water. The polymer produced is not a hazardous waste (as defined by United States Title 40 Code of Federal Regulations (40 CFR 261.24(a)). These neutralizing agents tend to reduce disposal costs and contribute to a safer work environment.

• In some cases after formaldehyde waste treatment with crystal products, the resulting solid waste may be discarded in approved laboratory solid waste streams. Before engaging in sewer disposal of neutralized formalin solutions, formal approval should be sought and received from the local waste water treatment authority.

• Wear appropriate protective gloves and protective clothing to prevent skin exposure. Protective eyeglasses or chemical safety goggles should be worn as
described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166 (19).

- Contact lenses should not be worn when working with formalin. Maintain ANSI-specification eye wash station and drenching facilities in work area.

6.2.8. Mercury-Based Fixatives

- Mercury's use in chemical analysis can be phased out in many cases, especially in Zenker's solution and histological fixatives. Some substitutes, such as copper, tin, zinc, and chromium compounds also have some risk, but less than that associated with mercury.

- Recycling companies may or may not accept mercury-containing waste, including laboratory solutions. The companies differ in the type of mercury waste accepted and the transportation requirements (6-8, 11-13, 15, 17, 18).

- Specific recommendations from the EPA include the following:
  - When possible, use alternative products that do not contain mercury.
  - Separate mercury-containing products before they get into the incineration waste stream.
  - Recycle mercury-containing products as much as possible to keep mercury out of the environment.
  - Source reduction is the preferred method for reducing mercury, but in some instances recycling can be implemented as an alternative.

6.2.9. Zinc-Based Fixatives (containing formalin)
Zinc-based fixatives are generally not considered a RCRA hazardous waste and are now accepted as good substitutes for those containing mercury-based compounds; these mercury-substitutes must not be disposed of with solid waste (5, 14).

Dispose of materials in accordance with federal (40 CFR 261), state and local requirements.

The recommended cleansing agent is water.

Zinc Formalin is toxic due to formaldehyde content. Dispose via a licensed waste hauler. Do not mix waste streams unless instructed to do so by your waste hauler. Some wastewater treatment authorities may grant permission for drain disposal of limited amounts if the zinc content is < 600 ppm.

Zinc Formalin is recyclable and can be neutralized with commercially available detoxification products.

Zinc-based fixatives are now available that do not contain formalin [Universal Fixatives: concentration, permanent stained smear, fecal immunoassays (with the exception of Entamoeba histolytica and the Entamoeba histolytica/E. dispar group that require fresh or frozen specimens for testing)]. Dispose of materials in accordance with federal (40 CFR 261), state and local requirements. The recommended cleansing agent is water.

6.2.10. Copper-Based Fixatives (containing no formalin)

Many localities restrict the amount of copper compounds that may be flushed down the drain.
• Ensure compliance with all government regulations.

6.2.11. Xylenes and Alcohols

• Xylene is categorized as a hazardous waste under RCRA and has been assigned EPA Hazardous Waste No. U239.

• Xylene also may be disposed of in an organometallic or organic lab pack that meets the requirements of 40 CFR 264.316 or 265.316. Xylene can be distilled using a variety of commercially available recycling units to produce reusable xylene.

• Xylene that cannot be saved for recovery or recycling should be handled as hazardous waste and sent to a RCRA approved incinerator or disposed in a RCRA approved waste facility.

• Processing, use, or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

• The disposal guidelines for alcohols (ethyl alcohol, isopropyl alcohol, methyl alcohol) are identical to those for xylene indicated above.

6.3. Working at the Bench

• In general, the same precautions and practices that are used in microbiology laboratories are relevant for those performing diagnostic parasitology procedures, particularly regarding aerosol transmission (2, 3).
Important guidelines for specimen collection and processing would include Standard Precautions, as well as the use of recommended equipment according to approved methods (biosafety cabinets, fume hoods, centrifuges, sharps, glassware, etc.) (2, 9, 10).

6.4. Personal Precautions

6.4.1. Biosafety Cabinet vs Fume Hood

- Although use of a fume hood is not mandatory when processing stool specimens containing formalin, an OSHA-compliant formalin monitoring program should be in use.

- Even with the substitution of dehydrating reagents other than xylene, fume hoods may be preferred in order to eliminate fecal and solvent odors. A small, table-top model is acceptable.

- A biological safety cabinet is not required for processing fecal specimens in the parasitology laboratory; however, some laboratories use Class I (open-face) or Class II-A2 (laminar-flow) biological safety cabinets for processing all unpreserved specimens.

- A biological safety cabinet is recommended if the laboratory is processing fresh specimens and performing cultures for parasite isolation (Table 6.1).

6.4.2. Personal Protective Equipment

- Use appropriate hand hygiene (washing and antiseptics) for laboratory work within a diagnostic parasitology laboratory.

- Disposable gloves of latex, vinyl, or nitrile can reduce exposure risk and should be worn during the accessioning and processing of all specimens for parasitologic examination, especially when handling blood, body fluids, and stool specimens (9, 10); this
recommendation applies whether the clinical specimens are fresh or are submitted in fecal preservatives.

6.4.3. Immunization

- All staff members with possible occupational exposure to the blood of humans and higher apes and body fluids must be offered the hepatitis B vaccine. Booster shots are no longer recommended by the CDC.

- Documentation, including signed statements and records of hepatitis B vaccination or declination, must be kept.

6.4.4. Disinfection

- General recommendations for the microbiology laboratory will be sufficient for use within the diagnostic parasitology section; these would include guidelines for disinfection of countertops, telephones, computers, equipment, and hands-free telephones.

6.4.5. Disposal of laboratory waste (special circumstances) (see Section 6.2)

- Fixatives containing mercury compounds:
  - Disposal of mercury compounds is severely restricted. Waste should be sent to an approved waste disposal facility. This guideline will apply to fecal collection vials and stain reagents that contain mercury (trichrome or iron hematoxylin alcohol/iodine dish).

  - The iodine in this staining dish removes the mercury and replaces it with iodine; therefore, when staining reagents are replaced, this dish must be placed in a container that can be sent to an approved waste disposal facility.
Mercury compounds are subject to reportable quantities under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and may not be flushed down the drain (www.atsdr.cdc.gov/cercla).

● Fixatives containing copper compounds:
  ○ Many localities restrict the amount of copper compounds that may be flushed down the drain.
  ○ Ensure compliance with all government regulations.

● Fixatives containing zinc compounds:
  ○ Many localities restrict the amount of zinc compounds that may be flushed down the drain.
  ○ Ensure compliance with all government regulations.

Fixatives containing formalin (sodium acetate-formalin-acetic acid): Incineration is the preferred disposal method for formaldehyde. Local governments often restrict the amounts of aldehydes that may be flushed down drain. Insure compliance with all government regulations.

6.5. Dirty vs Clean Areas of the Laboratory

● General guidelines for the microbiology laboratory will apply for the parasitology section of the laboratory. No special recommendations are necessary.

6.6. Instrumentation

● Safety requirements for the use of instruments would be the same as those used for a general microbiology laboratory and are primarily involved with specimen handling.

6.7. Antibody and Antigen Parasitology Testing
• Safety requirements for antibody and antigen testing would be the same as those used for a general microbiology or immunology laboratory and are primarily involved with specimen handling.
References


14. United States Environmental Protection Agency: (CFR 40, Part 261)


7. Mycology laboratory

7.1. Specimen receiving and log-in/set-up station
   7.1.1. Leaking containers
   7.1.2. Visual contamination on outside of container
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7.6. Select agents and pathogenic moulds

7.7. Blood culture bench

7.8. Instrumentation

7.9. Rapid testing (kits)

7.10. Molecular testing
7. MYCOLOGY LABORATORY

Although not a strict requirement, mycology laboratories that culture for filamentous fungi and manipulate those organisms should ideally be separate and isolated from the main microbiology laboratory with negative air pressure moving into the room from the main laboratory. Direct access to a Class II BSC is critical for this activity whether mycology work is conducted in a separate room or in an isolated section of the main laboratory. Most mycology diagnostic work can be conducted in the BSL-2 laboratory.

7.1. Specimen Receiving and Log-In/Set-Up Station

7.1.1. Leaking containers

● Guidelines for the general microbiology laboratory apply also for the mycology laboratory. No special recommendations are necessary.

7.1.2. Visual contamination on outside of container

● Guidelines for the general microbiology laboratory apply also for the mycology laboratory. No special recommendations are necessary.

7.1.3. Loose caps

● Guidelines for the general microbiology laboratory apply also for the mycology laboratory. No special recommendations are necessary.

7.2. Stains and Disposal

7.2.1. Gram stain
The Gram stain is not the optimum stain for fungus, but if used particularly for yeast, the same guidelines that apply to bacteriology/clinical microbiology should be followed for mycology.

7.2.2. Mycology stains

- Calcofluor white is not considered a hazardous substance; no special safety measures are required; use routine laboratory procedures, i.e., after skin contact, wash with soap and water; after eye contact, rinse eye well with water; seek medical attention in case of complaints after inhalation or swallowing.

- Follow accepted laboratory procedures applied to infectious materials for handling and disposing of slides.

- If potassium hydroxide (KOH) is used with the calcofluor white, more stringent precautions must be taken. See: Becton Dickinson MSDS for product # 261195, 2009 and refer to http://www.bdregdocs.com/msds/?ds=y&cnum=261195

  - Potassium hydroxide (KOH) 10-15 % solution is corrosive and should be handled with care; may cause burns or irritation to skin, eyes, and respiratory tract; avoid eye/skin contact and inhalation or ingestion; use gloves and eye protection if there is a danger of splashing or aerosol formation.

  - KOH is not listed as Resource Conservation and Recovery Act (RCRA) hazardous waste;

  - The small amount used on a slide with calcofluor white can be disposed of with slides containing infectious materials; if disposing of larger amounts, do so in accordance with federal, state and local requirements.
Lactophenol Cotton Blue (Lactophenol Aniline Blue or Poirrier's blue).

- In amounts commonly utilized in a clinical laboratory, lactophenol cotton blue is acidic, and care should be taken to avoid contact with the skin, eyes, and clothing.
- Rinse thoroughly with water if spilled.
- For fungal slides stained with the fluid, follow accepted laboratory procedures for handling and disposing of infectious materials.
- If larger amounts are to be produced or disposed of, the product is considered more hazardous; toxic by inhalation and contact with the skin, and especially if swallowed; use only with adequate ventilation.
- Dispose as hazardous waste in accordance with Federal, State, and Local Regulations.


● India Ink –

○ No special personal protection required under normal use conditions.

○ India ink is not a regulated hazardous waste. Dispose in accordance with applicable Federal, State, and Local Regulations.


● Acid-Fast Stain

○ Guidelines for the tuberculosis laboratory apply also for the mycology laboratory.

○ No special recommendations are necessary.

● Gomori Methenamine Silver Stain

○ Ingredients are toxic, corrosive, and harmful; avoid contact with skin and eyes.

○ Use with adequate ventilation; do not inhale.

○ Dispose of as a hazardous waste in accordance with applicable federal, state, and local regulations.


● Giemsa Stain
Due to the methanol content, it is toxic by inhalation, absorption, or ingestion; protective gloves and safety goggles are not required but are recommended.

Dispose of in accordance with federal, state, and local regulations. The preferred method is incineration via an approved facility.


7.3. Culture Reading at the Bench (1,2)

● A separate, closable room for mycology activities is recommended but not required.

● Conduct all culture manipulations in a BSC whether in a separate room or within a designated space in the open microbiology laboratory.

  ○ All mould colonies (filamentous, fuzzy, cottony, etc) must be handled in a Class II BSC. This applies as well to moulds growing on bacteriology plates. See section 7.6 for further information.

● Use shrink seals with petri plates (especially if mould begins to grow) in order to prevent accidental opening and spread of hyphal segments, conidia, or spores.

  ○ Never use Petri plates if *Coccidioides immitis* is suspected or if a filamentous culture is to be mailed or otherwise transported to another laboratory. Slants in screw-cap tubes should be utilized.

● Observe all plates and slants for growth before opening.
In general, cultures growing yeast-like colonies can be read on the open bench in a BSL-2 laboratory; but if the isolate is suspected of being *Cryptococcus neoformans* (moist, mucoid colonies) or any dimorphic fungus, it should be handled in a Class II BSC.

Never sniff a fungal culture to determine whether it has an odor. Plates containing molds should not be opened on the open bench, even if it is a bacteriology work station.

### 7.4. Personal precautions

#### 7.4.1. Biosafety Cabinet

- The Class II BSC is recommended for some mycology work, i.e. all moulds (i.e., fuzzy, wooly, cottony, powdery, or velvety) must be handled in the biosafety cabinet, never on the open bench.

- The same BSC guidelines that apply to bacteriology/clinical microbiology should be followed for mycology.

#### 7.4.2. PPE

- Guidelines for the general microbiology laboratory apply also for the mycology laboratory.

- Wear gloves when manipulating a mould culture having the possibility of being a dermatophye; when task is completed wash hands and wrists well (removing watches and bracelets).

#### 7.4.3. Disinfection

- Recommendations for the general microbiology laboratory are sufficient for use within the mycology laboratory; these include guidelines for disinfection of countertops, telephones, computers, equipment, and hands-free telephones, etc.
7.4.4. Decontamination and disposal of laboratory waste (waste management)

● The same guidelines that apply to clinical microbiology should be followed for mycology.

● If autoclave is unavailable and medical waste is handled offsite, plates and tubes containing *Coccidioides* should be opened and completely immersed in bleach overnight prior to disposal.

7.5. Dirty vs clean areas of the laboratory

● Guidelines for the general microbiology laboratory apply also for the mycology laboratory.

7.6. Select agents and pathogenic moulds

● Handle all mould-like colonies in a biosafety cabinet.

● Make a wet preparation of all cultured moulds before setting up a slide culture in order to detect structures that may indicate the possibility of the isolate being a highly pathogenic systemic fungus (2).

● Refrain from setting up slide cultures of isolates that on wet prep are suggestive of *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis, C. posadasii, Paracoccidioides brasiliensis, Penicillium marneffei*, or *Cladophialophora bantiana*. Make every attempt to identify them by well-prepared wet preps and DNA probes if available (2).

● If a laboratory-isolated organism is identified as *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides spp.*, or *Paracoccidioides brasiliensis*, BSL-3 practices and facilities are recommended for handling mould-form cultures and environmental samples likely to contain infectious conidia (1).
● *Coccidioides* spp. are the only fungi classified as a Select Agent requiring registration with CDC and/or USDA for possession, use, storage and/or transfer (1).

● To decontaminate *Coccidioides* isolates when autoclaving is not practiced in the laboratory (when culture plates are picked up by a contractor for off-site autoclaving), one could soak the plates, tubes, etc. overnight in bleach that completely immerses the opened tubes and plates prior to disposal.

### 7.7. Blood Culture Bench – for fungal requests

● Guidelines for the general microbiology laboratory apply also for the mycology laboratory.

● Plates growing mould-like colonies must be examined in a biosafety cabinet, not on the open bench.

● If a small-celled yeast (~3 μm) is seen, consider the possibility of *Histoplasma capsulatum*, and handle under BSL-2 conditions in a Class 2 BSC (2).

### 7.8. Instrumentation

● Instruments used for mycologic studies are most commonly those for continuously monitored blood culture and for yeast identification. The same guidelines that apply to bacteriology/clinical microbiology should be followed for mycology.

### 7.9. Rapid Testing (kits)

● The same guidelines that apply to clinical microbiology should be followed for monomorphic yeasts in mycology
• If the isolate is a mould, it must be handled in the BSC.

7.10 Molecular Testing

The same safety guidelines that apply to clinical microbiology should be followed for mycology with the additional rule that mould isolates must be handled in a BSC during extraction of nucleic acids.

References:


8 Virology laboratory

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8.1.1 Biohazards associated with specimen receiving and log-in
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8.3 Handling cell cultures at the bench

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8. Virology Laboratory

8.1 Specimen processing and login bench

8.1.1 Biohazards Associated with Specimen Receiving, Log-In and Set-Up Stations

- The clinical virology laboratory receives a wide variety of clinical specimens for virus detection. Because the infectious nature of this material is largely unknown, special care must be taken to prevent contamination of personnel, the environment, and other clinical specimens.

- Handle all clinical specimens under BSL-2 conditions. (1) Some special pathogens and select agents must be handled under more stringent biosafety conditions.

- Wear a laboratory coat, gloves, and eye protection whenever clinical specimens are handled. This includes the specimen receiving and login processes.

- Conduct culture setup and all other specimen manipulations in a class II or higher BSC.

- Compile a “Do Not Process/Do Not Test” list containing the names of suspect agents that should not be opened or processed (e.g., smallpox, Ebola virus, vesicular stomatitis virus, foot and mouth virus, swine fever viruses, CDC Select Agents). Such specimens received for testing need to be promptly and properly
packaged and shipped to an appropriate reference laboratory. Contact the appropriate reference laboratory in advance of any forwarding.

- If the laboratory has a BSL-3 facility, the laboratory should also compile a list of suspect agents that must be processed and tested under BSL-3 conditions.

### 8.1.2 Leaking containers

- Do not process leaking containers as they can be a hazard to the technologist, could contaminate the laboratory, or present an opportunity for specimen co-mingling and/or contamination that could produce a false result.

- Inform the attending physician or submitting veterinarian or organization as to why testing will not be performed and a new specimen should be requested.

- Place leaking specimens into a biohazard bag and decontaminate by autoclaving or another approved method.

### 8.1.3 Visual contamination on outside of container

- Specimens with a small amount of contamination (e.g., a dried blood spot) on the outside of the container should be brought to the attention of the laboratory director. The director can examine the specimen and determine if it is suitable for testing and whether it constitutes a hazard to laboratory personnel.

### 8.1.4 Special precautions for suspicious specimens
● Accept specimens transported to the laboratory by enforcement officials in accordance with local and state regulations and following chain of custody procedures. However, the purpose of the testing and the suspect agent should be determined before testing is started. Appropriate procedures to ensure chain of custody should be in place and followed even if the sample is not processed.

● Establish a laboratory suspect agent “Do Not Process/Do Not Test” list. The specimen login and processing personnel are responsible for checking the suspect agent list before the specimen is sent on for testing. Notify the laboratory director if the suspect agent is on the Do Not Process/Do Not Test list.

● If the laboratory has a BSL-3 facility, the specimen processing and login personnel should have a list of tests and suspect agents that must be handled only in the BSL-3 laboratory.

● Do not process or test unapproved or unusual specimen types as described in the laboratory accessioning SOP.

● Do not accept any specimen that the laboratory is not certified to test.

8.2 Stains, Chemicals, and Disposal

● Chemical hazards in the virology and electron microscopy laboratory will vary depending upon the extent of service provided by the laboratory. The potential hazards presented in this section are intended to be illustrative, not inclusive of all
chemicals used, and educational in nature and should not replace Material Safety Data Sheet (MSDS) information or state, local, or institutional policies.

- The chemical fume hood used in the laboratory must be certified annually and checked daily for appropriate operation parameters.

### 8.2.1 Alcohols

- Ethanol, methanol, isopropyl alcohol, and alcohol blends are used in the Virology Laboratory to fix cells, for nucleic acid extraction and precipitation, and as a disinfectant.

- Store concentrated alcohols in a cabinet rated for flammable storage. Isopropyl and methanol alcohol vapors can be toxic and these products should only be used in well-ventilated areas. Isopropyl alcohol can also cause contact dermatitis. Alcohols are effective disinfectants for enveloped viruses but they have little effect on non-enveloped viruses.

- Alcohols are volatile and should not be used in closed spaces. Exposure to solvent fumes can cause eye, nose and throat irritation, drowsiness, headaches, and skin dryness. When possible, use these in a fume hood.

- Do not use alcohols around open flames or instruments that cause sparks.

- Wear latex, vinyl, or nitrile gloves when handling alcohols to minimize skin exposure.
• Some alcohols will cloud plastics and care must be taken when wiping down plastic instrument faces with alcohols.

8.2.2 Antibiotics

• Antibiotics in routine use include penicillin, streptomycin, gentamicin, ciprofloxacin, kanamycin, tetracycline, Amphotericin B, and neomycin. These antibiotics can be found in culture media and viral transport media.

• Concentrated antibiotic mixtures are frequently used to increase the antibiotic concentrations in samples containing large numbers of bacteria or fungi. Concentrated antibiotic solutions can be purchased at 50 times (50X) and 100 times (100X) the working concentration.

• While the risks associated with antibiotic preparation and usage are relatively low in the virology laboratory, antibiotic preparation and handling has been associated with hypersensitivity reactions (2;3), asthma, (3-7) and contact dermatitis in hospital, pharmaceutical, and animal workers.

• Aerosolized antibiotic liquids and powders can serve as sensitizing agents and they may precipitate hypersensitivity reactions in antibiotic-sensitive individuals.

Antibiotic Safety.

• Always wear gloves, mask, and eye protection when handling antibiotic powders and when preparing or dispensing concentrated antibiotic
solutions. Respiratory protection (e.g. fume hood, mask, or positive pressure respirator) may be required in some instances.

- Care should be taken to prevent aerosol generation when working with antibiotic powders and solutions as these can contaminate the environment, sensitize other laboratory workers (8), and could present a hazard to antibiotic-sensitive individuals (3;9).

- Laboratory personnel with known antibiotic sensitivities should not prepare concentrated antibiotic solutions.

- It may also be prudent to exclude pregnant employees from preparing concentrated antibiotic solutions because antibiotics may have adverse or unknown effects on the developing fetus.

8.2.3 Bleach Solutions (see 3.4.2)

8.2.4 Cycloheximide

- Cycloheximide is used as an antibiotic, protein synthesis inhibitor, and a plant growth regulator. In the virology laboratory, cycloheximide is used in Chlamydia re-feed media.

- Cycloheximide powders and solutions are irritants, causing redness, itching and burning. Animal studies have shown that cycloheximide causes adverse reproductive effects including birth defects, sperm toxicity, and testicular damage. It is not known if cycloheximide can cause similar
reproductive effects in humans but it should be handled as if it were a reproductive toxin. The user should consult MSDS documents for more information.

- The highest potential for exposure in the laboratory is during the weighing of cycloheximide powders and during the preparation of cycloheximide solutions. Cycloheximide may enter the body as an aerosol, and orally through dust exposure. Exposure can also occur through hand contamination of food, beverages, cosmetics, or directly by touching the mouth with contaminated hands.

- Handle cycloheximide powder in a chemical fume hood.

- Wear personal protective equipment including laboratory coat and gloves when handling cycloheximide powders and solutions in order to prevent skin contamination, skin absorption, and/or hand-to-mouth exposure. Wash hands with soap and water after glove removal.

- **Cycloheximide disposal.** Cycloheximide is inactivated by alkaline solutions (pH > 7.0). Aspirating cycloheximide-containing culture fluids into vacuum traps containing 10% bleach will inactivate the chemical. Most soaps and detergents are alkaline and these agents will also inactivate cycloheximide.

8.2.5 Dimethyl Sulfoxide (DMSO)
● Dimethyl sulfoxide is used as a cryoprotectant when freezing cell cultures. DMSO is a powerful solvent and can penetrate skin and latex gloves.

● Minimize contact with skin and mucus membranes.

● Wear laboratory coat and eye protection when handling DMSO solutions. Double gloving may be prudent as the chemical will eventually penetrate latex gloves.

● Nitrile gloves, which are commonly used in chemical laboratories, are rapidly dissolved by DMSO.

● DMSO easily penetrates the skin, and substances dissolved in DMSO may be quickly absorbed. This property has been used as a drug delivery system to allow antifungal medications to penetrate skin, toenails and fingernails. In the laboratory however, DMSO exposure could facilitate the absorption of contaminants. When DMSO comes into contact with the skin, some people report that they can quickly taste an oyster- or garlic-like flavor.

● DMSO is mutagenic for mammalian somatic cells, bacteria, and /or yeast. Chronic exposure may cause damage to blood, kidneys, liver, skin, mucus membranes, and eyes. See MSDS documents for more information.

8.2.6 Electron Microscopy Stains, Fixatives, and Buffers
• **Osmium** tetroxide and gluteraldehyde are used as electron microscopy fixatives. Liquid and vapor components are strong fixatives and will quickly fix the skin, mucus membrane and eye tissues of laboratory personnel.

  ○ Open vials in the chemical fume hood. Keep vials in double bottles and seal the tops with parafilm.

  ○ Handle vials with disposable gloves.

  ○ Use eye protection, gloves, and disposable laboratory coats when handling the fixative and when fixing tissues.

• **Uranyl acetate, phosphotungstic acid, and ammonium molybdate** are used as negative stains in the electron microscopy laboratory. All of these compounds contain heavy metals and are very toxic if inhaled, ingested or introduced through cuts or abrasions. Uranyl acetate is weakly radioactive and powders should be kept in a metal container. Phosphotungstic acid is corrosive and causes burns on exposed skin and mucous membranes. Ammonium molybdate is very dangerous in case of eye contact, ingestion, and inhalation.

  ○ Laboratory workers must use personal protective equipment including laboratory coat, gloves, and eye protection when handling powders and solutions. See MSDS documents for more information.
○ Prepare these stains in a chemical fume hood to prevent inhalation.

○ Care must be taken to prevent contamination of work areas with powders.

Electron microscopy buffers such as sodium cacodylate and veronal acetate contain arsenic and sodium barbital, respectively. These buffers must be handled with caution.

○ Prepare buffers in a chemical fume hood to prevent inhalation of powders.

○ Use personal protective equipment including laboratory coat, gloves, and eye protection when handling powders and solutions.

See MSDS documents for more information.

8.2.7 Electron Microscopy embedding media

● (Meth)acrylates and epoxy-based materials are frequently used to embed biological samples for electron microscopy. Epoxy products include Epon, Araldite, Spurr resin, and Maraglas. Formvar (polyvinyl formal) is used as a support film for EM grids and for making replicas. Many of these compounds are toxic, carcinogenic or potentially carcinogenic and
are known to cause skin irritation, dermatitis, and skin sensitization. The user is directed to the individual MSDS documents for more information.

- Use these compounds in well ventilated areas, preferably in a chemical fume hood. Many of these chemicals are dissolved in flammable solvents and they should be kept away from heat and ignition sources.

- Plastic monomers will quickly penetrate latex and vinyl gloves so change these gloves frequently when embedding with plastics (10-13).

- Wear gloves when handling or trimming plastic embedded blocks. All of the monomers may not be polymerized and unpolymerized monomers will retain their toxic properties.

- Cover working areas with paper towel or plastic-lined absorbent pads and clean up spills immediately with alcohol.

- Use soap and water to remove any resins that come into contact with skin. Do not use alcohol to remove resins from skin because alcohol increases penetration of the resin.

- **Disposal of embedding media and film-making solutions.** Embedding materials are generally less hazardous when polymerized or hardened.
○ Never pour plastic containing solutions (e.g., propylene oxide-epon mixture) down the drain. They will harden in the drain and can plug it.

○ Harden all waste before disposal. Store hardened waste in a fume hood and dispose of the container as hazardous waste.

○ Store discarded containers, beakers, vials, pipettes, etc. that have been in contact with resins or support films in puncture-resistant containers in the fume hood until they can be sent off as hazardous waste.

8.2.8 Ethidium Bromide

● Ethidium bromide (EtBr) is a DNA intercalating agent that is commonly used as a non-radioactive marker for visualizing nucleic acid bands in electrophoresis and other gel-based separations. EtBr is a potent mutagen, toxic after acute exposure, and is an irritant to the skin, eyes, mouth and the upper respiratory tract.

○ Handle pure EtBr in a chemical fume hood because the powder can easily contaminate the entire laboratory.

○ Designate an area where EtBr work is going to be performed and only use EtBr solutions in that area.
Cover surfaces within the designated area with plastic-lined absorbent pad. The pad should be replaced on a scheduled basis or when it becomes contaminated.

Use personal protective equipment including lab coat, eye protection and gloves when handing EtBr solutions and gels. Note: Latex gloves provide little protection against EtBr. Nitrile gloves provide an effective short-term barrier. Double gloving provides increased protection.

Wash hands thoroughly after de-gloving.

Application of sodium hypochlorite solutions to spent solutions of ethidium bromide will deactivate the ethidium bromide, but the reaction products are mutagenic, according to the Ames test. (82) Use an alternative deactivation method, use or a permitted hazardous waste treatment facility to dispose of these spent solutions.

Use of sodium hypochlorite solutions in work areas of ethidium bromide use is also not recommended.

**EtBr waste management.** Collect and manage even small volumes or concentrations of EtBr waste as hazardous waste or follow local regulations.
○ Bag materials coming into contact with EtBr and dispose of as hazardous chemical waste.

○ Minimize EtBr solution volumes by reacting them with activated charcoal. The charcoal can be collected by filtration and placed into leak-resistant containers for hazardous waste disposal.

○ Place agarose gels containing EtBr into a leak-resistant plastic container and dispose as hazardous waste.

8.2.9 Evan’s Blue

● Evan’s Blue is used as a counterstain during fluorescence microscopy. Evan’s Blue powders and solutions are skin irritants but there is no known flammability, carcinogenicity, or teratogenicity warning associated with this compound.

● The highest potential for exposure in the laboratory is during the weighing of Evan’s Blue powders and during the preparation of solutions. Breathing powders can cause respiratory irritation. Skin and mucus membrane irritation can also occur.

● Handle Evan’s Blue powder in a chemical fume hood to prevent inhalation.

● Wear personal protective equipment including laboratory coat, eye protection, and gloves when handling Evan’s Blue powders and solutions in order to prevent skin contamination.
8.2.10 Guanidinium Solutions

- Guanidinium chloride, guanidinium thiocyanate, and guanidium isothiocyanate are chaotropic agents used to disrupt cells and denature proteins (particularly RNases and DNases) during nucleic acid extraction procedures.

- Handle guanidinium powders in a chemical fume hood to prevent inhalation.

- Wear personal protective equipment including laboratory coat, gloves, and eye protection when handling powders and solutions.

- These chemicals are strong irritants and eye exposure can result in redness, irritation and pain. They are toxic if ingested and may cause neurological disturbances. If inhaled, guanidinium compounds can cause respiratory tract irritation coughing, and shortness of breath.

- Do not add bleach to any sample waste containing guanidinium thiocyanate due to the production of toxic fumes. Guanidinium compounds are reactive with acids and other oxidizers producing toxic fumes including cyanide vapors (thiocyanate and isothiocyanate derivatives), hydrochloric acid vapors (guanidinium hydrochloride), and nitrogen oxides (all forms).

8.2.11 Neutral Red
Neutral red is a pH indicator and a vital stain used in some plaque assays. It may be harmful if swallowed, inhaled, or absorbed through the skin and can cause irritation to the skin, eyes, and respiratory tract.

Handle neutral red powder in a chemical fume hood to prevent inhalation.

Wear personal protective equipment including laboratory coat and gloves when handling neutral red powders and solutions in order to prevent skin contamination.

The highest potential for exposure in the laboratory is during the weighing of neutral red powders and during the preparation of solutions.

8.2.12 Merthiolate (Thimerosal)

Merthiolate or thimerosal is a mercury-containing antiseptic and antifungal agent used as a preservative in some laboratory solutions.

Concentrated thimerosal is very toxic by inhalation, ingestion, and in contact with skin.

Wear personal protective equipment including laboratory coat and gloves when handling merthiolate powders and solutions in order to prevent skin and mucous membrane exposure.
- The low quantities used in some commercial reagents are relatively safe but thimerosal exposure can have cumulative effects. In the body, merthiolate is metabolized or degraded to ethylmercury \((\text{C}_2\text{H}_5\text{Hg}^+)\) and thiosalicylate. Ethylmercury clears from blood with a half-time of about 18 days, and from the brain in about 14 days.

8.2.13 Organic Solvents

- Acetone is the principal organic solvent used in the virology laboratory and is primarily used as a fixative for cell smears.

- Acetone is flammable and it is classified as an irritant causing eye damage, skin and respiratory tract irritation. Chronic exposure can result in reproductive, nervous system, kidney, liver and skin damage.

- Store acetones in a flammable storage cabinet and keep away from sources of heat, sparks, or flame. Do store or use acetone in a refrigerator that is not rated as explosion proof. Sparks from the refrigeration pump and the door actuated light switch could ignite acetone fumes and cause an explosion.

- Use acetones in a well-ventilated area (or chemical fume hood) to prevent respiratory irritation.

- Do not use vinyl exam gloves for handling acetones and other aggressive organic solvents because vinyl gloves can be dissolved by these agents.
● Latex gloves will eventually dissolve in acetone and double gloving is recommended for short-term acetone usage.

● Acetones will dissolve or cloud many plastics and care must be taken to protect plastic devices from acetone exposure.

● **Electron Microscopy**

  ● Many volatile solvents including ethane, propylene oxide, and ethers are used in the electron microscopy laboratory. These solvents are extremely flammable and are fire and explosion hazards. Care must be taken to prevent static discharges that could ignite the chemicals.

  ● Use these solvents in a chemical fume hood to prevent respiratory irritation and minimize the buildup of explosive vapors.

  ● Wear personal protective equipment including laboratory coat, eye protection, and chemically resistant gloves when handling these chemicals.

  ● It may be prudent to disconnect flammable gas lines to electron microscopy laboratories to discourage the use of open flames.

  ● Store small quantities in a well-ventilated flammable storage cabinet and keep these reagents away from sources of heat, sparks, or flame.
8.2.14 Sodium Azide

● Sodium azide is a common preservative in many laboratory reagents including monoclonal antibodies, buffers, and enzyme immunoassay reagents.

● Sodium azide is an acute toxin and a mutagen. All contact with this substance should be reduced to the lowest possible level.

● Sodium azide and hydrazoic acid (HN₃, which is formed from NaN₃ in water) are known to produce hypotension (low blood pressure) in laboratory animals and humans, and to form strong complexes with hemoglobin, thereby blocking oxygen transport in the blood.

● Wear personal protective equipment including gloves, lab coats, and eye protection when handling solutions containing sodium azide.

● Sodium azide is not explosive except when heated near its decomposition temperature (300°C) or reacted with metals.

● Never flush solid or concentrated sodium azide solutions down the drain since this practice can cause serious incidents when the azide reacts with lead or copper in the drain lines and explodes.

● Dilute solutions (0.1%) found in most laboratory reagents may be flushed down the sink with copious volumes of water to prevent metal-azide build up. Sodium azide reacts with heavy metals (such as silver,
gold, lead, copper, brass, or solder in plumbing systems); and metal salts to form highly explosive compounds such as lead azide and copper azide. These metal azides can explode when the plumbing is repaired or jarred.

● A skin designation has been assigned to the OSHA Permissible Exposure Limits due to the ability of sodium azide to readily penetrate intact skin. Any dermal exposure can significantly contribute to the overall exposure to sodium azide.

● Sodium azide is not compatible and may react violently with chromyl chloride, hydrazine; bromine; carbon disulfide; dimethyl sulfate; and dibromomalonitrile; strong acids (such as hydrochloric, sulfuric and nitric); and acid chlorides.

### 8.3 Handling cell cultures at the bench

● All cell cultures, whether inoculated with clinical specimens or not, are potentially infectious. Unintended or adventitious viral agents have been found in many cell lines, diploid cells, and primary cultures (Table 1). Adventitious agents may be spread during cell culture manipulations and can originate from:

  ○ Latently or persistently infected primary tissue, secondary cultures, and cell lines.

  ○ Animal products such as fetal calf serum, trypsin, etc.

  ○ Transforming agents (HPV, SV-40, herpesviruses, retroviruses, adenoviruses, etc.) used to immortalize cells.
Many of these agents do not produce cytopathic effects and cell passage and archiving can perpetuate these agents for generations.

**8.3.1 Cell lines**

- Primary cultures of human (and potentially animal) tissue, cells, and blood present the greatest risk for harboring unintended or adventitious infectious agents that can infect humans. As a result, OSHA included human cell lines in its Final Rule on Bloodborne Pathogens. Even though OSHA CFR 29, Bloodborne Pathogens refers to human blood and tissue, adherence to it in the veterinary laboratory is advisable. Tissue culture procedures should be done in a Class II BSC.

- Primate cells and tissues also present risks to laboratory workers. SV-5 and SV-40 are common contaminants of primary rhesus monkey kidney cells and cultures from macaques and other Old World monkeys may be latently infected with Herpesvirus simiae (B-virus). B-virus infection presents an often fatal hazard for personnel handling these animals and their tissues.

- Cultures from nude and severe combined immune deficient (SCID) mice pose a special risk of harboring agents that could cause silent, chronic infections

- Some cell lines were immortalized with viral agents such as SV-40, EBV adenovirus or HPV. These cells may produce infectious virus or they may have viral genomic material within the cells. Other cell lines may carry viral genetic elements that were introduced purposefully during experiments or inadvertently during culture manipulation. These viruses and virus genetic elements survive
freezing and may be present in archived culture materials. Many cell lines are also persistently infected with broad host range retroviruses that can present an infection hazard for laboratory workers.

- Tumorigenic human cells may present a potential hazard due to self-inoculation. (14-17) Tumors or tumor cells that have been inoculated into nude mice may acquire additional adventitious agents including lymphocytic choriomeningitis virus (LCMV).

- Many of the adventitious viruses do not produce cytopathic effects or alter the cell phenotype and many can survive freezing and storage in liquid nitrogen for long periods.

- When infected tumor cells are cultured, the supernatant fluids can contain infectious virus and the laboratory may optionally elect to treat this as biohazardous waste. LCMV presents a special problem for pregnant women as the virus can be transmitted to the fetus causing fetal death or serious central nervous system malformation.
### Table 1. Selected adventitious agents associated with cell cultures, organs, and tissues that could be used to generate cell cultures, and cell culture reagents.

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Source</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Immunodeficiency Virus (HIVs)</td>
<td>Blood cells, serum, plasma, solid</td>
<td>(29)</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>Human kidney, pancreas, some adenovirus transformed cell lines, rhesus monkey kidney cells</td>
<td>(18-22)</td>
</tr>
<tr>
<td>Lymphocytic</td>
<td>Multiple cell lines, mouse tissue</td>
<td>(34-36)</td>
</tr>
<tr>
<td>Bovine rhinotracheitis virus</td>
<td>Bovine serum, fetal bovine serum</td>
<td>(23)</td>
</tr>
<tr>
<td>Bovine rhinotraceitis virus (LCM)</td>
<td>(significantly lower risk today due to ultrafiltration of bovine serum)</td>
<td>(37)</td>
</tr>
<tr>
<td>Mycoplasmas</td>
<td>Many cell cultures</td>
<td>(39)</td>
</tr>
<tr>
<td>Parainfluenza type 3</td>
<td>Monkey kidney cells</td>
<td>(21;38)</td>
</tr>
<tr>
<td>Myxovirus (SV5)</td>
<td>Fetal porcine kidney cells, trypsin</td>
<td>(21;22;49)</td>
</tr>
<tr>
<td>Bovine enterovirus</td>
<td>Bovine kidney, liver ixial vessel conduit</td>
<td>(21;22;49)</td>
</tr>
<tr>
<td>Porcine parvovirus</td>
<td>Kidney, human foreskin, monkey</td>
<td>(21;22;24)</td>
</tr>
<tr>
<td>Bovine herpesvirus</td>
<td>Rhesus, cynomologous, and African green monkey kidney cells</td>
<td>(21;50)</td>
</tr>
<tr>
<td>Rabies virus</td>
<td>Human cornea, kidney, liver, iliac vessel conduit</td>
<td>(40-49)</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>Kidney, human foreskin, monkey</td>
<td>(21;22;24)</td>
</tr>
<tr>
<td>Simian adenoviruses</td>
<td>Human kidney cells</td>
<td>(21;50)</td>
</tr>
<tr>
<td>Epstein-Barr Virus</td>
<td>Some lymphoid cell lines and tissues</td>
<td>(25)</td>
</tr>
<tr>
<td>Simian Foamy Virus (FHV)</td>
<td>HVS, cynomologous, and human kidney</td>
<td>(21;22;38;51)</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
<td>Human blood, liver</td>
<td>(26)</td>
</tr>
<tr>
<td>Virus/Microorganism</td>
<td>Cells/Tissues/Components</td>
<td>References</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Simian Virus 40 (SV40)</td>
<td>Rhesus monkey kidney cells</td>
<td>(23;52;53)</td>
</tr>
<tr>
<td>Simian Viruses 1 through 49</td>
<td>Rhesus monkey kidney cells</td>
<td>(21;23;51)</td>
</tr>
<tr>
<td>Swine torque teno virus</td>
<td>Trypsin, swine-origin biological components</td>
<td>(54)</td>
</tr>
<tr>
<td>Squirrel monkey retrovirus</td>
<td>Multiple cell lines, commercial interferon preparations</td>
<td>(55;56)</td>
</tr>
<tr>
<td>West Nile Virus</td>
<td>Human blood, heart, kidney, liver, lung, pancreas</td>
<td>(57-77)</td>
</tr>
</tbody>
</table>

### 8.3.2 Cell Culture Practices

- Workers who handle or manipulate human or animal cells and tissues are at risk for possible exposure to potentially infectious latent and adventitious agents that may be present in those cells and tissues.

- Recommended cell culture practices from the CDC/NIH publication “Biosafety in Microbiological and Biomedical Laboratories, 5th edition” (1) include:
  - Strictly follow BSL-2 recommendations for personnel protective equipment such as laboratory coats, gloves and eye protection.
  - Handle human and other primate cells using BSL-2 practices and containment.
○ Perform all work in a Class II or higher BSC. Class I safety cabinets and clean benches should never be used for cell culture manipulation because they do not provide adequate protection for the operator.

○ Autoclave or disinfect all material coming into contact with cell cultures before discarding.

○ Enroll all laboratory staff working with human cells and tissues in an occupational medicine program specific for bloodborne pathogens and work under the policies and guidelines established by the institution’s Exposure Control Plan.

○ Laboratory staff working with human cells and tissues should provide a baseline serum sample (if this is institutional policy based on a risk assessment), be offered hepatitis B immunization and should be evaluated by a health care professional following an exposure incident(1). Similar programs for work with non-human primate blood, body fluids, and other tissues are recommended.

8.3.3 Biohazards Associated with Cell Culture Reading

● Dried medium on the lip of culture tubes could present a contamination hazard. Handle all culture vessels as if they were contaminated.

○ Wear gloves and a lab coat when handling viral cultures.

○ Use eye protection if there is a potential splash hazard.

○ Perform all culture manipulations in a BSC.
• Decontaminate culture tubes and other materials that come into contact with cell cultures using an effective method (usually autoclaving or chemical disinfection) before disposal. (1)

• Shell vial, tube, and other cultures go through multiple manipulations (media aspiration, re-feeding, inoculation, etc.) that can generate aerosols. These aerosols and manipulations can contaminate the outside of the culture vessel.

• The occasional leaking tube on another shelf could also contaminate the outside of vessels on lower shelves and may not leave visible evidence of the contamination.

### 8.3.4 Biohazards Associated with Liquid Nitrogen Usage.

Liquid nitrogen can become contaminated when ampoules are broken in the dewar and contaminants can be preserved in the nitrogen (78). (Sections 3.9.6) These potentially infectious contaminants can contaminate other vials in the dewar and generate an infectious aerosol as the liquid nitrogen evaporates. Plastic cryotubes rated for liquid nitrogen temperatures are recommended for liquid nitrogen storage because they appear to be sturdier than glass ampoules and are less likely to break in the nitrogen. Glass ampoules are not recommended.

Ampoules and cryotubes can explode when removed from liquid nitrogen creating infectious aerosols and droplets. See the section on Cryotube Explosions (3.9) for more information.
8.4. Personal precautions

No amount of safety engineering can reduce the physical, chemical, and biological risks in a laboratory environment if personal precautions are not employed consistently and rigorously. All laboratory workers and visitors are responsible for following established procedures regarding personal precautions. Directors and supervisors should refer to the CDC/NIH publication “Biosafety in Microbiological and Biomedical Laboratories, 5th edition” (1) to review their biosafety responsibilities.

8.4.1. Biological Safety Cabinet (BSC)

All culture manipulations in the virology laboratory should be performed in a class II BSC. These manipulations include, but are not limited to culture inoculation, feeding, passage, hemadsorption and hemagglutination testing, virus dilutions and titrations, cell fixation, immunofluorescent staining, and preparing controls and control slides. Cytocentrifuge and other sealed centrifuge heads containing specimens or cultures should be opened in the BSC to prevent the spread of infectious aerosols.

BSC Operation and Setup Notes. An extensive BSC review can be found in the CDC/NIH publication “Biosafety in Microbiological and Biomedical Laboratories, 5th edition” (1).

- Class II-A1 is appropriate for work that does not use volatile chemicals. Class II-A2 can be canopy connected for use with small amounts of volatile chemicals.
Class II-B2 may be appropriate when volatile chemicals might also be used. Refer to Section 3, Table 1.

- Certify BSCs according to NSF/ANSI Standard 49 annually, or after cabinet is moved, HEPA filters are replaced or disinfected, any major repairs that could affect the seating or performance of the HEPA filtration system.

**BSC Disinfection.** Do not use alcohols as a primary disinfectant in BSCs because alcohols have little or no effect on non-enveloped viruses and vapors from isopropyl alcohol and isopropyl alcohol can be toxic and may cause contact dermatitis. A 10% bleach solution provides the best disinfecting activity but care must be taken to remove the residual chlorine with water as the chlorine will eventually corrode the stainless steel surfaces.

- Remove the contents of the BSC and disinfect the interior of the BSC daily or after a spill or contamination event.
- Remove the floor plate of the cabinet and the front grate monthly or bimonthly for cleaning. Disinfect the floor plate, grate, and the plenum below the floor plate as described below.
- Wipe work surfaces, interior walls and the interior surface of the of the window with a 10% solution of household bleach (1) followed by one wiping with water to remove the residual chlorine and one wiping with 70% ethanol (EtOH). Remove residual chlorine because it will eventually corrode stainless steel surfaces.
- Wipe down any items that will be returned to the BSC.
Let the blower run for at least 4 minutes to remove any particulates.

Some laboratories leave the sash up and blowers running at all times while other laboratories turn off the blower and close the sash (if so equipped) at the end of the day.

8.4.2. Personal Protective Equipment (PPE)

Refer to Section 3 for PPE discussion.

Vinyl exam gloves are not recommended for handling acetones and other aggressive organic solvents because the gloves can be dissolved by these agents (Sections 3.2 and 8.2.13).

Gloves must be worn to protect hands from exposure to hazardous materials and extreme temperatures. In the molecular biology area, gloves are also used to protect the specimen from nucleases that are on the skin.

Gloves must be worn when

- Handling specimens (Section 8.1.1)
- Handling cultures (see Sections 3.2, 8.3.3, and 8.3.2)
- Handling nucleic acids and amplification reagents
- Working with hazardous chemicals (Section 8.2)
- Working in a biological safety cabinet (Section 8.4.1)
- Whenever there is an opportunity for contaminating the skin with hazardous materials.
• Remove gloves and wash hands when work with hazardous materials has been completed and before leaving the laboratory.

• Do not wash or reuse disposable gloves.

• Never touch your face, mouth, eyes, or other mucus membranes when wearing gloves in the laboratory.

• Because gloves are potentially contaminated, they should be placed into biohazard disposal containers.

• Do not wear gloves outside the laboratory or in public areas such as library, rest rooms, cafeterias, administrative areas, and hallways.

**Laboratory coats** are used to protect the clothing from hazardous materials. Protective, fluid-resistant laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials. (1) (Section 3.2.2)

• Ensure that front-opening laboratory coats are closed when handling specimens, cultures, working in a biological safety cabinet, and any other activities that could result in splashes and clothing contamination.

• Remove protective clothing before leaving for non-laboratory areas (e.g., cafeteria, rest rooms, library, and administrative offices).

• Place disposable laboratory coats into medical waste containers for disposal.

• Laboratory clothing should not be taken home. If the laboratory elects to launder laboratory coats, the coats should be deposited for laundering by the institution.

**Eye and face protection** (goggles, mask, face shield and/or other splatter guards) is used when handling specimens, cultures, and anytime that splashes or sprays of infectious or
other hazardous materials are anticipated. Splash and spill precautions include but are not limited to the following procedures.

- Eye and face protection (goggles, mask, face shield or other splatter guard) must be used whenever a splash or spray event is anticipated. This includes, but is not limited to opening, pipetting, manipulating, or testing specimens, cultures, biological agents, or other hazardous materials outside of the biosafety cabinet.
- If eye and face protection becomes contaminated, decontaminate them before reuse or disposal with other contaminated laboratory waste.
- Contact lenses are not considered personal protective equipment. Laboratory workers who wear contact lenses must also use face protection as described above. In a chemical splash, contact lenses can intensify eye damage because the lens will hold the chemical against the eye for a longer period of time.
- If a laboratory worker wearing contact lenses receives a splash to the eye, the eye must be flushed with water, the lens should be removed, and the eye should be flushed again. Disposable contact lenses should be discarded. Re-usable contact lenses should be disinfected before being returned to the eye.

### 8.4.3 Disinfection

Fingers and bench tops are frequently contaminated during laboratory manipulations, unrecognized spillage, and by airborne organisms. These organisms can be picked up by the fingers, pens, pencils, eye or safety glasses, and other items and transferred to the mouth or eyes. Disinfection of the environment is an important means for reducing exposure to and spread of infectious agents in the laboratory.
- Hand washing protocols for laboratory workers and visitors must be rigorously followed and enforced.

- To prevent contamination of ungloved hands, the laboratory should be designed so that it can be easily cleaned.
  - Decontaminate work surfaces with an appropriate disinfectant (usually 10% household bleach) after completion of work and after any spill or splash of potentially infectious material.
  - Utilize bench tops that are impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
  - Cover chairs used in laboratory work with a non-porous material that is easily cleaned.
  - Carpets and rugs are not appropriate in laboratories. (1)
  - Ergonomic anti-fatigue mats are essentially rugs and should be discouraged in the virology laboratory. Reasons for avoiding these mats include the following:
    - They make it difficult to clean up spills.
    - They make it difficult for the custodial staff to clean and disinfect the floors.
    - These mats may place custodial staff at increased risk if they pick up or move mats that have been inadvertently contaminated with chemicals or infectious agents.
    - Liquids will often wick under the mat, hiding potential contamination problems.
- Mats impede cart movement in the aisle and could cause culture vessels or other objects to fall off the cart.
- Mats can cause laboratory chairs to tip when chairs bump into the edge of the mat. This action could cause workers to drop or bump into culture vessels as they try to regain their balance.
- Mats can be a trip hazard that could impede emergency egress from the laboratory in an emergency.
  - Avoid clutter in the laboratory. Boxes and papers are difficult to disinfect and may mask inadvertent spills or aerosol contamination.
  - Telephones are a convenient vehicle for transferring infectious agents to the face and mucus membranes.
    - Never pick up or dial a telephone with gloved hands.
    - Disinfect telephones regularly. Note: Alcohols do not inactivate non-enveloped viruses or destroy DNA.
    - Use the hands-free or speaker phone features whenever possible to avoid touching the telephone handset to the face.
  - Do not bring briefcases, purses, backpacks, books, magazines, and other personal items into the laboratory. These items are difficult to disinfect and contamination (especially aerosol contamination) may not be noticed. When these items are taken home they could transfer infectious agents to friends and family members.
  - Because computers are used for logging in specimens, recording results, and other specimen and culture related activities, computer keyboards
should be considered dirty areas and they should be disinfected regularly.

Hands should be washed after using the keyboard with ungloved hands.

8.5. **Decontamination and disposal of laboratory waste** (waste management)

Disinfect reagents or materials coming into contact with clinical specimens, cell cultures, or virus cultures (including gloves and personal protective equipment) before being placed into the medical waste stream. Fluids may be disinfected by treating with 10% household bleach (Section 3.4.2 and 3.5) or by autoclaving. Disinfect all other materials by autoclaving before they are placed into the medical waste.

Decontaminate specimens, reagents, cultures and equipment that comes into contact with specimens from patients with suspected Creutzfeldt-Jacob or other prion-associated disease according to local protocols, usually by autoclaving for 1 hour in the presence of 20,000 ppm hypochlorite solutions or 1 N NaOH (1).

8.6. **Clean vs. dirty areas of the laboratory**

In the virology laboratory, the distinction between dirty and clean areas is a misnomer as all areas within the laboratory present increased opportunities for encountering infectious, chemical, and physical hazards. Laboratory precautions described in this chapter should be followed in all areas of the laboratory.
Cell culture preparation and reagent preparation areas are often called “clean” areas because no specimens, amplified nucleic acids, or control materials are allowed in these areas. The goal of establishing and policing these “clean” areas is to prevent reagent and cell culture contamination that could produce false results. These are laboratory areas and laboratory precautions still apply.

8.7. Early recognition of high-risk organisms

Section 3 emphasizes the importance of the recognition of possible agents of bioterrorism and other organisms causing a public health emergency. Many of these trigger events are listed in Table 2 below, and would have been investigated in any case as a quality assurance function and the “typical cause” listed in Table 2 will be the actual cause in nearly every case. Laboratory technologists and directors should, however, be aware that other high risk causes are possible. How the laboratory responds to these trigger events will depend upon whether they have a BSL-3 facility and the capabilities of their state and local laboratory response network.
Table 2. Trigger events requiring supervisor or laboratory director intervention.

<table>
<thead>
<tr>
<th>Event</th>
<th>Typical Causes</th>
<th>High-Risk Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimen Processing/Login Bench</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specimen brought in by law enforcement official with a chain of custody form</td>
<td>Assault case workup</td>
<td>Potential BT/BC specimen</td>
</tr>
<tr>
<td>Suspect agent is on the Do Not Test/Do Not Process list</td>
<td>Clerical mistake at order entry</td>
<td>Could be from a patient with a high-risk infection.</td>
</tr>
<tr>
<td>Unusual (non-biological) or unapproved specimen type or container</td>
<td>Clerical mistake at order entry Inappropriate order</td>
<td>Powders, environmental samples, animal specimens, clothing, food samples, inanimate objects could contain BT/BC agents. Could represent an attempt to insinuate a BT/BC agent into the laboratory.</td>
</tr>
<tr>
<td><strong>Fluorescent Antibody Bench</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dim staining when controls and other positive specimens stain normally</td>
<td>Decreased antigen expression in cells</td>
<td>Cross-reaction with another infectious agent.</td>
</tr>
<tr>
<td>Unusual staining pattern</td>
<td>Added wrong antibody to well</td>
<td>Altered virus or</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Antibodies ran together during incubation</th>
<th>genetic/antigenic variant.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-reaction with another agent.</td>
<td></td>
</tr>
<tr>
<td>Staining unusual cell types</td>
<td></td>
</tr>
<tr>
<td>Added wrong antibody to well</td>
<td>Infectious agent with altered host range</td>
</tr>
<tr>
<td>Antibodies ran together during incubation</td>
<td></td>
</tr>
<tr>
<td>Cross-reaction with another agent.</td>
<td></td>
</tr>
</tbody>
</table>

**Cell Culture Bench**

<table>
<thead>
<tr>
<th>CPE Pattern and cell tropism is unusual</th>
<th>Unusual/unexpected pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT/BC agent in specimen</td>
<td></td>
</tr>
<tr>
<td>Familiar CPE pattern but in an unusual cell type</td>
<td>Genetic drift</td>
</tr>
<tr>
<td>Unusual/unexpected pathogen</td>
<td></td>
</tr>
<tr>
<td>BT/BC agent in specimen</td>
<td></td>
</tr>
<tr>
<td>Unexpected CPE/HAd pattern from the indicated specimen source.</td>
<td>Clerical error when entering source</td>
</tr>
<tr>
<td>Unusual/unexpected pathogen</td>
<td></td>
</tr>
<tr>
<td>BT/BC agent in specimen</td>
<td></td>
</tr>
<tr>
<td>Confirmation protocols do not work</td>
<td>Genetic or antigenic drift</td>
</tr>
<tr>
<td>Unexpected/unusual pathogen</td>
<td></td>
</tr>
<tr>
<td>BT/BC agent in specimen</td>
<td></td>
</tr>
<tr>
<td>Nucleic Acid Testing</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Altered melting curve shape, too many peaks, altered $T_M$ when controls and other patient curves are normal</td>
<td></td>
</tr>
<tr>
<td>Genetic variation in agent</td>
<td></td>
</tr>
<tr>
<td>Poor extraction</td>
<td></td>
</tr>
<tr>
<td>Primer dimers</td>
<td></td>
</tr>
<tr>
<td>Unusual/unexpected pathogen</td>
<td></td>
</tr>
<tr>
<td>More than one agent present</td>
<td></td>
</tr>
<tr>
<td>Chimeric, recombinant or reassortant pathogen</td>
<td></td>
</tr>
<tr>
<td>Change in the slope of the amplification curve</td>
<td></td>
</tr>
<tr>
<td>Genetic variation in agent</td>
<td></td>
</tr>
<tr>
<td>Poor extraction</td>
<td></td>
</tr>
<tr>
<td>Specimen inhibition</td>
<td></td>
</tr>
<tr>
<td>Unusual/unexpected pathogen</td>
<td></td>
</tr>
<tr>
<td>Chimeric, recombinant or reassortant pathogen</td>
<td></td>
</tr>
</tbody>
</table>

8.7.1 Fluorescent antibody testing bench.

- Dim staining of cells when control smears and other positive specimens stain strongly can be due to poor antigen expression, sampling crusted lesions, and cellular degradation. Dim staining can also occur when the antibody reagent cross-reacts with a similar antigenic epitope arising from antigenic drift, the presence of a viral subspecies, or an unsuspected virus. This type of reaction occurs more frequently when staining with polyclonal antibodies.
• An unusual pattern of staining (e.g., speckled cytoplasmic staining with a reagent that usually produces nuclear staining) can indicate the presence of an unsuspected virus.

• Staining in an unusual cell type (e.g., staining of squamous cells rather than ciliated respiratory epithelial cells) could indicate the presence of a different virus or an expected virus with an altered tropism.

• These findings should be brought to the attention of the laboratory supervisor or director.

8.7.2 Suspicious or unusual results

• Known agents of bioterrorism or public health emergencies such as foot and mouth disease, flaviviruses, smallpox, alpha viruses, and hemorrhagic fever viruses will grow in routine cell cultures used in the clinical laboratory. If a culture produces a CPE pattern that is consistent with one of these agents, do not open for further testing. Contact the attending physician or veterinarian for more information on the patient/animal and contact the state or local Laboratory Response Network (LRN) laboratory for further instructions.

• Cultures that produce a familiar CPE pattern in an unusual cell type and/or an unexpected CPE result from a specific specimen source (an eye specimen producing hypertrophic rounding in nearly every cell type) may indicate the presence of an altered or unexpected virus. Contact the physician or veterinarian for more information and move the specimen into the BSL-3 laboratory for any additional workup.
Confirmatory test failure can be caused by antigenic drift or operator error during testing. Alternative confirmatory tests such as PCR can be used because PCR methods generally present fewer risks than additional fluorescent antibody staining. If PCR testing fails to identify the virus, the culture should be moved into the BSL-3 laboratory for further workup.

If a BSL-3 laboratory is not available, the laboratory should contact their state or local LRN laboratory for assistance in identifying the viral agent.

8.7.3 Nucleic Acid Testing

Specimens that produce an altered melting curve shape, too many peaks, and/or an altered $T_M$ when controls and other patient curves are normal, could indicate poor extraction, the presence of primer dimers, or genetic changes under the primers or probes. These results can also be caused by the presence of an unexpected or unusual (chimeric, recombinant or reassortant) pathogen. Re-extraction and re-testing presents a modest additional risk. If the results continue to be unusual, the specimen may be referred to the LRN laboratory for additional testing.

Likewise, PCR specimens with altered amplification slopes may be the result of genetic variations under the primers and/or probe or the presence of an unusual pathogen. Re-extraction and re-testing presents a modest additional risk. If the results continue to be unusual, the laboratory may elect to refer the sample to their state or local LRN for additional characterization.
8.8 Hazards Associated with the Electron Microscopy Laboratory

Diagnostic electron microscopy can be a relatively simple and rapid method for morphologic identification of agents in a specimen. Electron microscopy procedures can serve as a general screen to detect novel organisms or organisms that have altered genetic or immunologic properties that render them undetectable by nucleic acid or immunoassay protocols (79). Electron microscopy laboratories share many of the physical, chemical, and biological hazards described for the virology laboratory but some unique situations should be discussed.

8.8.1 Flammable and Combustible Liquids. The Electron microscopy laboratory uses a wide variety of flammable solvents and the use of open flames is discouraged. (Section 8.2.13)

- Place solvents requiring refrigeration in special flammable storage refrigerators that minimize exposed electrical connections and reduce the opportunity for spark generation.
- Store flammable liquids in flammable storage cabinets in accordance with local and state regulations.
- Handle flammable liquids in fume hoods to minimize vapor buildup. Ultrasonic cleaning of Wehnelt cap assemblies in an acetone baths must be done in a chemical fume hood.
- Never pour flammable liquids down the drain as they can cause an explosion.
8.8.2 X-Ray Hazards. The electron microscope will generate dangerous levels of X-rays within the microscope as high energy electrons strike the metal components. Modern electron microscopes have sufficient shielding and lead impregnated glass viewing ports that minimize the dangers to the operator. However, modifications to the instrument, adding and removing accessories, and some maintenance procedures can compromise the shielding.

A calibrated thin-window GM survey meter should be used to verify the shielding effectiveness and monitor radiation levels

- Initially at the time of installation,
- Whenever the microscope is modified, and
- Periodically as indicated by state, local, or institutional policies.

Radiation levels should be less that 0.5 mR at 5 centimeters from the unit.

8.8.3 Electrical Hazards. Information regarding routine electrical safety in the EM Laboratory can be found in Section 3.7. Special high voltage and high amperage electrical sources are used to power electron microscopes and other equipment in the laboratory. This equipment should be serviced by trained technicians. Safety interlocks and power lockouts should be installed to prevent activation of electrical circuits while the instrument is being serviced.
- Changing the filament in the high-voltage electron gun can present electrical hazard if the grounding rod does not make contact with the Wehnelt cap. A significant charge can build up on the cap and the charge must be relieved before touching the cap.

8.8.4 Chemical Hazards. A number of heavy metal stains and aggressive fixatives are used in the electron microscopy laboratory. More information about these hazards can be found in Section 8.2.6 and in the MSDS materials provided by the manufacturers. Embedding and film making materials (Section 8.2.7) are chemical hazards and many of these materials are dissolved in flammable organic solvents (Section 8.2.13).

- Limit acute and chronic exposure to these chemicals.
- Place embedding ovens in a chemical fume hood to minimize exposure to potentially hazardous chemicals.
- Latex gloves are not appropriate for all chemicals and appropriate glove usage must be emphasized for all laboratory personnel.

8.8.5 Cryogens and Compressed Gases. The most commonly used cryogens used in the electron microscopy laboratory are liquid nitrogen and liquid helium. The hazards associated with liquid gasses are discussed in Section 3.9. Compressed helium, CO₂ and nitrogen are also used in the EM Laboratory. Hazards and safety measures associated with these gases are summarized in Section 3.9.
8.8.6 Specialized Equipment. The electron microscopy laboratory uses a number of specialized instruments that can be hazardous to use. For example, evaporators, freeze-driers, freeze-fracture, and sputter coater units use vacuum and the vessels could implode. The implosion hazards are reviewed in Section 3.13.1.

- To prevent eye damage during evaporation, use welder’s goggles to view the source.
- Allow the components to cool before touching them
- Do not breathe in any of the evaporated metal that may flake off from the surface.

8.8.7 Critical point dryers can be quite dangerous because of the high pressures generated within the “bomb” (10;80). Follow the operating and safety procedures described in the operator’s manual for safe operation.

- Place a one-half inch thick lexan shield between the operator and the bomb.
- Do not secure the shield directly over the window of the bomb where it would receive the full force of an explosion. That force could shatter the shield.
- A polycarbonate face shield is also recommended when observing the contents of the bomb.
- Vent freons or freon substitutes that vent from critical point dryers directly outside or through a fume hood.
8.8.8 Biological Hazards. The biological hazards of the electron microscopy laboratory are similar to those of the virology laboratory and good laboratory practices must be followed.

- Conduct primary specimen handling in a biological safety cabinet to prevent aerosols and contamination of the laboratory.
- Negative staining solutions may not inactivate microorganisms and viruses.
  - Osmium tetroxide is an effective sterilant but it cannot be used for all specimens.

8.9. Rapid Testing (kits)

A number of FDA-approved rapid immunodiagnostic tests (RIT) for viral antigens and antibodies are available at this writing. Originally designed for point of care or near point of care testing, many of these tests are being used for testing in clinical virology laboratories. The following biosafety recommendations are based upon the CDC interim biosafety guidance document for handling 2009-H1N1 specimens published August 15, 2009. (81)

Procedures that involve only simple steps such as inserting a swab into medium or pipetting specimens and reagents and are not reasonably expected to generate aerosols may be performed on the bench top using only splash protection. Testing procedures that require centrifugation, vortexing, or vigorous mixing, or other methods that could generate aerosols should be performed in Class II biological safety cabinet.
Perform bench top testing in a manner that will prevent splashes and the generation of aerosols. The appropriate personal protective equipment for this type of testing would include a laboratory coat, gloves, eye protection and a facemask such as a surgical, dental, medical procedure, isolation, or laser mask. A splash shield providing protection of the entire face fulfills the need for separate eye protection and facemask.

Decontaminate work surfaces and equipment with a 10% bleach solution as soon as possible after specimens are processed.

Rapid immunodiagnostic testing, when performed in a Class II biological safety cabinet, does not require the use of additional of eye protection and a facemask.

8.10 Hazards Associated with the Molecular Virology Laboratory

Molecular virology laboratories share many of the physical, chemical and biological hazards described for the virology laboratory they also present some unique hazards.

8.10.1 Electrical Hazards. Information regarding routine electrical safety in the molecular virology Laboratory can be found in Section 3.7. Special high voltage power sources are used in electrophoresis and nucleic acid sequencing equipment.

- Never use high voltage electrical equipment near flammable liquids and gases because an arc could cause an explosion or fire.
- Disconnect the power to electrophoresis equipment before disassembling the apparatus.
- Only trained technicians should service high voltage electrical equipment.
• Never tamper with or defeat safety interlocks and power lockouts on electrophoresis equipment.

8.10.2 Ultraviolet (UV) Light Hazards. Short wave UV view boxes are often used to visualize nucleic acid bands in gels. Special care must be taken to protect eyes and completely cover the skin when visualizing and photographing gels on a UV view box.

• Wear gloves, long-sleeved lab coat, and a UV-resistant full face shield when working with UV view boxes.

• Only use face shields rated for short wave ultraviolet light for this purpose.

• Locate UV view boxes out of the normal traffic flow of the laboratory so that bystanders or people passing by are not exposed to the ultraviolet light. Other ultraviolet light hazards are discussed in Section 3.12.

8.10.3 Chemical Hazards. The chemical hazards unique to the molecular virology laboratory include chloroform, ethidium bromide (Section 8.2.8) and guanidinium-based extraction reagents (Section 8.2.10). Acute and chronic exposure to these chemicals should be avoided.

8.10.4 Biological Hazards. The biological hazards in the molecular virology laboratory are similar to those of the virology laboratory and good laboratory practices must be followed.
• Conduct primary specimen handling in a biological safety cabinet to prevent aerosols and contamination of the laboratory.

• Extracted nucleic acids may or may not be sterile. A poor extraction procedure may not completely remove or inactivate the infectious agents in the specimen. It has been reported that the genome of positive-stranded RNA viruses (poliovirus) is infectious and once introduced into the cell, the viral genome requires no virus-coded proteins or accessory components for virus replication. While the risks of infection in this manner are very small, it is prudent to handle extracted viral nucleic acids as if they were infectious. This practice mirrors specimen handling procedures used in other areas of the laboratory and supports a unified specimen handling policy for the entire laboratory.
Reference List


9. **Chemistry Laboratory**

9.1. Automated analyzers

9.2. Tissue preparation for chemical/toxicological analysis

9.3. Specific analyzer risks

9.3.1. Graphite furnaces

9.3.2. Mass spectrometer
9. CHEMISTRY LABORATORY (INCLUDING TOXICOLOGY AND DRUG TESTING)

All human and animal-origin specimens tested by the chemistry, toxicology, or drug testing laboratory may contain infectious agents and it is imperative to understand and minimize the risk of exposure to patient specimens through surface contact, aerosolization, or penetrating injury. Users are referred to the section on risk mitigation of laboratory acquired infections that are addressed in Section 2 and 3.

9.1 Automated Analyzers (see also 3.17.3 and 10.6.3)

Automated analyzers frequently have added features to help reduce operator exposures but do not totally eliminate potentials for exposure. A common feature in newer systems is closed system sampling.

- Chemistry/toxicology analyzers often have high velocity robotic arms and samplers that might cause skin punctures and lacerations.
  - Only operate analyzers with the cover closed.

- Sample probes that move quickly or deliver fluid rapidly may generate aerosols and droplets.

- Always use instruments according to manufacturer instructions.

- Ensure instrument safety shields and containment devices are in place at time of use.
  - Limit the amount of hand movement near the sample probe and liquid-level sensors.
On instruments where the operator is required to wipe sample probes after sampling, wear disposable gloves and use gauze pads with impermeable plastic coating on one side.

- Newer instruments have automatic probe wash cycles eliminating this source of exposure.

- To prevent spillage, handle sample trays and samples with caution and keep them covered when not being manipulated.

- Assume that the outside of blood tubes are contaminated and be prepared to wipe the outside of the tube with the laboratory disinfectant or with a solution of 1:10 household bleach.

- Fill sample cups and aliquot tubes using mechanical devices (i.e. transfer pipettes) and never decant (pour) them.

- Consider effluents of clinical analyzers contaminated with pathogens and some may also be contaminated with hazardous chemicals. Their disposal should comply with state and local regulations. Investigate these effluents and consult applicable standards before discharging them in the sewer.

- Follow manufacturer instructions for routine cleaning and trouble-shooting specimen spills on or within an instrument, including the appropriate PPE and type of cleaning solution to be used.

  - When manufacturer instructions do not include spill containment and clean up instructions, collaborate with manufacturer to develop an SOP that will effectively protect the operator and maintain and extend the instrument’s operational life.
• Have fresh 1:10 household bleach on hand in case of an emergency spill or breakage after being assured by the manufacturer that chlorine will not damage instrument components.

• Perform daily cleaning of fluidic systems and sampler following manufacturer instructions.

• Collect waste into a waste container that contains fresh concentrated household bleach in sufficient quantity to achieve a final concentration of 10% bleach when the flask is full. Effluents containing compounds not compatible with bleach (Figure 1) should not be collected into bleach to avoid formation of chlorine gas.

9.2 Tissue preparation for chemical/toxicological analysis

• Consider all unfixed tissues as biohazardous, regardless of the patient diagnosis or the test(s) ordered.

• The use of a fixative is not always sufficient to eliminate all types of biohazards.

• Use a BSC or PPE including gown and gloves with a fixed containment device for sample aspiration.

• Automated sample loading systems reduce sample handling and also perform sample vortexing in an enclosure that prevents operator exposure to aerosols and splashes.
  o Take special care when loading samples onto the instrument tube racks.
  o For unfixed samples, load instrument tube racks inside a biosafety cabinet or wear PPE to protect from splashes and aerosols.
9.3 Specific Analyzer Risks

To fully assess the risk of active biohazards in analyzer effluents or processes, risk analysis should begin with assessment of procedures that occur prior to utilization of specific analyzers. Sample preparation protocols may fully inactivate viruses and bacteria so that the risk of biohazardous aerosol generation in the analyzer effluent is essentially zero. One example is the use of protein-precipitation techniques or protein denaturing solvents in liquid chromatography that would negate biohazard concerns in aerosols or effluents generated by the analyzer.

9.3.1 Graphite Furnaces

- Completely dry samples before vaporization.
- Adequately ventilate devices that heat vaporizes specimens to assure that infectious agents are not escaping into ambient air.
- Keep instrument covers and panels closed and secure while instrument is in use in accordance with manufacturer’s recommendations.

9.3.2 Mass spectrometers

- When mass spectrometers are used as detection devices that are programmed to monitor selected ions from the effluent of liquid chromatography (LC-MS), there could be exposure to infectious agents in the effluent if pre-analyzer process risk assessment shows that sample preparation procedures do not inactivate infectious agents.
- Devices that use nondestructive (soft) techniques to ionize samples, e.g. sonic spray ionization, may present a risk of exposure to operators if pre-analyzer process risk assessment shows that sample preparation procedures do not inactivate infectious agents.
Figure 1. List of compounds incompatible with household bleach (sodium hypochlorite)

**Sodium Hypochlorite Incompatibility Chart**

Do **NOT** mix Sodium Hypochlorite (bleach) with **ANY** other chemical unless adequate engineering controls and personal protective equipment (PPE) are in place. Accidental mixing may cause dangerous conditions that could result in injury to personnel and/or damage to property or the environment.

<table>
<thead>
<tr>
<th>Incompatible Material</th>
<th>Mixing May Result In</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids, Acidic Compounds and Acid Based Cleaning Compounds such as:</td>
<td>- Release of chlorine gas, may occur violently.</td>
</tr>
<tr>
<td>- Alum (Aluminum Sulfate)</td>
<td>- Hydrochloric Acid (HCl)</td>
</tr>
<tr>
<td>- Aluminum Chloride</td>
<td>- Sulfuric Acid</td>
</tr>
<tr>
<td>- Ferrous or Ferric Chloride</td>
<td>- Hydrofluoric Acid</td>
</tr>
<tr>
<td>- Ferrous or Ferric Sulfate</td>
<td>- Fluorosilic Acid</td>
</tr>
<tr>
<td>- Chlorinated Solutions of Ferrous Sulfate</td>
<td>- Phosphoric Acid</td>
</tr>
<tr>
<td>- Brick and Concrete Cleaners</td>
<td>- Brick and Concrete Cleaners</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemicals and Cleaning Compounds containing ammonia such as:</td>
<td>- Formation of explosive compounds.</td>
</tr>
<tr>
<td>- Ammonium Hydroxide</td>
<td>- Release of chlorine or other noxious gases.</td>
</tr>
<tr>
<td>- Ammonium Chloride</td>
<td>- Ammonium Sulfate</td>
</tr>
<tr>
<td>- Ammonium Silicofluoride</td>
<td>- Quaternary Ammonium Salts (Quats)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic Chemicals and Chemical Compounds such as:</td>
<td>- Formation of chlorinated organic compounds.</td>
</tr>
<tr>
<td>- Solvents and Solvent Based Cleaning Compounds</td>
<td>- Formation of explosive compounds.</td>
</tr>
<tr>
<td>- Fuels and Fuel Oils</td>
<td>- Release of chlorine gas, may occur violently.</td>
</tr>
<tr>
<td>- Amines</td>
<td>- Propane</td>
</tr>
<tr>
<td>- Organic Polymers</td>
<td>- Ethylene Glycol</td>
</tr>
<tr>
<td>- Insecticides</td>
<td>- Methanol</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Metals such as:</td>
<td>- Release of oxygen gas, generally does not occur violently. Could cause overpressure/rupture of a closed system.</td>
</tr>
<tr>
<td>- Copper</td>
<td>- Cobalt</td>
</tr>
<tr>
<td>- Nickel</td>
<td>- Iron</td>
</tr>
<tr>
<td>Avoid piping and material handling equipment containing stainless steel, aluminum, carbon steel or other common metals.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>- Release of oxygen gas, may occur violently.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Reducing agents such as:</td>
<td>- Evolution of heat, may cause splashing or boiling.</td>
</tr>
<tr>
<td>- Sodium Sulfite</td>
<td>- Sodium Hydrosulfite</td>
</tr>
<tr>
<td>- Sodium Bisulfite</td>
<td>- Sodium Thiosulfate</td>
</tr>
</tbody>
</table>

The Chlorine Institute has available for $25 a 30-minute videotape, Handling Sodium Hypochlorite Safely. Pamphlet 96, Sodium Hypochlorite Manual, also is available. See the "Publications" section of the Institute's Internet web site, www.CL2.com, for ordering information or contact the Publications Department, 202-775-2790.
10. Hematology and phlebotomy laboratory

10.1. Specimen receiving and log-in/set-up station
   10.1.1. Leading containers
   10.1.2. Visual contamination on outside of container
   10.1.3. Loose caps

10.2. Work at the open bench
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   10.3.1. Biosafety cabinet
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10.5. Clean vs dirty areas of the laboratory

10.6. Instrumentation
   10.6.1. Waterbaths
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   10.6.3. Automated hematology/hemostasis analyzers
   10.6.4. Flow cytometers
   10.6.5. Automated slide strainers
   10.6.6. Total or semi-automated hematology test systems

10.7. Rapid testing (kits)

10.8. Molecular testing

10.9. Phlebotomy
   10.9.1. Sharps workbook
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   10.9.3. Clean vs dirty areas in the laboratory
   10.9.4. Pneumatic tube systems
   10.9.5. Personal precautions
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10.9.7. Disinfection patient room work areas and drawing stations
10.9.8. Equipment decontamination
10.9.9. Documentation of training and competency assessment
10.9.10. State and other regulations
10.10.

10. Hematology Laboratory (Including Hemostasis and Flow-Cytometry)

10.1 Specimen Receiving and Log-In/Set-Up Station

10.1.1 Leaking containers
   ● See 3.1.1

10.1.2 Visual contamination on outside of container
   ● See 3.1.2

10.1.3 Loose caps
   ● See 3.1.3

10.2 Work at the open bench

10.2.1 Operational SOPs
   ● See 3.1.4

10.2.2 Manual removal of sealed caps and specimen aliquotting/pipetting
   ● See 3.1.5

10.2.3 Unfixed Specimens
   ● Microscopes:
     ○ Disinfect the stage, eyepieces, knobs and any other contaminated parts daily.
     ○ Select a disinfectant that will be non-corrosive to the microscope and appropriate for potential infectious agents.

   ● Slide preparations –
     ○ Avoid waving slides in the air or use of electric fans at an open bench to air dry slides.
○ Use of an electric fan to air dry slides should be in a BSC or fume hood. Keep in mind that the use of a fan in the A2 BSC will disrupt airflow and will render the BSC unsuitable for other laboratory work until the airflow has been stabilized.

● Buffy coat smears

○ Disposable Wintrobe tubes should be used in place of capillary tubes to avoid cutting glass tubes and possible exposure to blood and shards of glass.

● Hemacytometer

○ Use extreme caution when using glass hemacytometers and glass coverslips to avoid punctures from glass shards.

  • Plastic hemacytometers are commercially available and offer repeatable and reliable measurements and analysis.

  • Several hemacytometer designs eliminate the use of coverslips and allow for exact volume control.

● Bleeding Times (CLSI Performance of the Bleeding Time Test - H45A2E)

○ Wear gloves – see 3.2.2 and 8.4.2.

○ Use appropriate pediatric/adult template safety devices.

○ Ensure devices are reviewed annually in compliance with OSHA Bloodborne Pathogens Standard (last amended December 12, 2008).

available resources) and


○ Use disposable razors when removal of arm hair from test site is required.

○ Dispose of used bleeding time device and razor in sharps container and filter paper in medical waste container.

● Bone marrow aspirates.

○ Include appropriate hospital infection control policies and procedures for patient care settings in the laboratory policy and procedure for collection of bone marrow aspirates when collected in these settings.

○ Limit what is brought into patient rooms.

  • Bone marrow safety tray kits are marketed by several manufacturers.

  • Use of disposable trephine and aspiration needles are recommended.

○ Collect specimens and prepare squash smears and slides in the laboratory.

○ If smears must be prepared outside the laboratory, e.g. in patient care settings, use Standard Precautions; use risk and hazards assessment to determine what/when additional PPE might be appropriate; use a flat secure surface to prepare smears and slides; ensure cover/caps on additional specimens are tightly secured before transport; and ensure slides, squash smears, and additional specimens are transported in leak proof and breakage-resistant receptacles.

● Document training and assess competency.
Include knowledge of, and adherence to, hospital infection control policies/procedures in patient settings and the concept of Standard Precautions in all documented training and competency assessments.

10.3 Personal precautions
   See 3.2

10.3.1 Biosafety Cabinet
   ● Class II biosafety cabinet is required for all aerosol-generating processes.
   ● See 3.3

10.3.2 Personal Protective Equipment
   ● See 3.2.1

10.3.3 Disinfection
   ● See 3.4

10.4 Decontamination and disposal of laboratory waste- (regulated medical waste management) (CLSI Clinical Laboratory Waste Management (GP05A2E))
   ● See 3.5

10.5 Dirty vs. clean areas of the laboratory
   ● See 3.16

10.6 Instrumentation
Whether automated or manual, procedures with the potential for producing specimen aerosols and droplets (e.g. stopper removal, vortexing, opening or piercing evacuated tubes, automatic sample dispensers, etc.) require either PPE or engineering controls designed to prevent exposures to infectious agents. See 3.17

10.6.1 Waterbaths

- See 3.17.1

10.6.2 Centrifuges

- See 3.17.2

10.6.3 Automated Hematology/Hemostasis Analyzers

- Automated analyzers frequently have added features to help reduce operator exposures but do not totally eliminate potentials for exposure. A common feature in newer systems is closed system sampling. See also 3.17.3, 9.1, and 11.6.3

- Sample probes that move quickly or deliver fluid rapidly may generate aerosols and droplets.

- Always use instruments according to manufacturer instructions.

- Ensure instrument safety shields and containment devices are in place at time of use.

- Limit the amount of hand movement near the sample probe and liquid-level sensors.

- Wear gloves and use gauze pads with impermeable plastic coating on one side on instruments where the operator is required to wipe sample probes after sampling.
○ Newer instruments have automatic probe wash cycles eliminating this source of exposure.

○ Handle sample trays and sample plates with caution and cover them when not being sampled to prevent spillage.

○ Fill sample cups and aliquot tubes using mechanical devices and never decant (pour) them.

○ Assume that effluents of clinical analyzers are contaminated and disposal should comply with applicable federal, state, and local environmental regulations.

○ Follow manufacturer instructions for routine cleaning and trouble-shooting specimen spills on or within an instrument, including the appropriate PPE and type of cleaning solution to be used.

○ When manufacturer instructions do not include spill containment and clean up instructions, collaborate with manufacture to develop an SOP that will effectively protect the operator and maintain and extend the instrument’s operational life.

10.6.4 Flow cytometers (See 3.17.3)

● Occupational exposures in a routine flow cytometry (FCM) laboratory arise either from sample handling or more specifically from aerosols and droplets generated by the flow itself. Flow cytometric applications, e.g., phenotypic analysis, calcium flux evaluations, apoptosis measurements of unfixed cells, when performed using jet-in-air flow cytometers with extremely high pressure settings can expose operators to potentially hazardous aerosols.
FCM biosafety procedures should specifically focus on aerosol containment, waste management and equipment maintenance. (Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells. H43-A2. Wayne, PA. CLSI 2007)

Consider all unfixed materials (peripheral leukocytes, bone marrow, various body fluids, cultured cells and environmental samples) as biohazardous.

○ The use of a fixative is not always sufficient to eliminate all types of biohazards.

○ Use a BSC or PPE with an equivalent fixed containment device for sample aspiration.

○ Disposable gloves and protective clothing should always be worn by flow cytometer operators.

○ Only documented-trained and experienced operators (professional consensus is 2 years) should perform potentially biohazardous cell sorting. (See International Society for Analytical Cytology Biosafety Standard for Sorting of Unfixed Cells, January 2007).

○ Cell-sorters are equipped with a nozzle to form a jet of microdroplets: this step is likely to generate aerosols.

○ Instrument failures such as clogged sort nozzle or air in the fluidic system can drastically increase aerosol formation.
○ Restrict access to essential personnel during sorting. Post a notice at the entrance that cell sorting is in process. Personnel wishing to monitor the sort must wear the same personal protective gear as the operator of the cell sorter.

○ Newly designed safety attachments for cell sorters have become commercially available.

○ Some enclosed fluid system flow cytometers perform cell sorting using a fluid switching mechanism. These cytometers, in contrast to jet-in-air cell sorters, do not generate aerosols during cell sorting.

○ Efficiency of aerosol control measures on sorter instruments should be tested periodically following manufacturer instructions, particularly when unfixed human cells and known biohazardous samples are acquired or sorted.

○ Simpler bead-based technology for measuring efficiency have been developed.

● Newer flow cytometers have added biosafety features for reduced risk of operator exposure to instrument-generated sample droplets and aerosols, e.g., enclosed flow cells, droplet containment modules, automated samplers.

● Ensure the instrument is used in a manner specified by the manufacturer.

● Open and close the instrument cover with care.

● Keep instrument covers and panels closed and secure while instrument is in use.

● Do not defeat safety interlocks and/or sensors.
● Place sample tubes securely into the sample introduction port, otherwise it could be blown off once it is pressurized and splash sample onto the operator.

○ Some sample ports contain a metal sip tube that can pose a risk of damaging gloves when the tube is not inserted carefully.

○ For improved splash protection, it is recommended that safety glasses or chemical splash goggles be worn during acquisition.

○ Automated sample loading systems reduce sample handling and also perform sample vortexing in an enclosure which prevents operator exposure to aerosols and splashes.

    • Take special care when loading samples onto the instrument tube racks.

    • For unfixed samples, load instrument tube racks inside a biosafety cabinet or wear PPE to protect from splashes and aerosols.

● Perform daily cleaning of fluidic systems and sampler following manufacturer instructions.

● Collect waste into a waste container that contains fresh concentrated household bleach in sufficient quantity to achieve a final concentration of 10% when the container is full.

● Note - Adding chlorine to a waste container where ammonium chloride tris buffer is used as a lysing agent may release dangerous chlorine gas (use a broad-spectrum idophor instead).

10.6.5 Automated slide stainers

● Ensure the instrument is used in a manner specified by the manufacturer.
• Keep instrument covers and panels closed and secure while instrument is in use.

10.6.6 Total or semi-automated hematology test systems

• Hazard and risk assessments should be conducted to identify critical operations that pose a risk for exposure.

• Operational SOPs should include:
  ○ Instructions for troubleshooting tube breakage and specimen spills on conveyor tracts, belts, sorter, aliquot, and cap-piercing probe stations.
  ○ Instructions requiring at least daily cleaning and disinfection and cleaning after tube breakage and/or specimen spills.

  • If instructions are not provided by the manufacturer then collaborate with the manufacturer to develop trouble-shooting and cleaning procedures that will protect the operator and be compatible with and extend the life of the robotic equipment.

  • Identification of appropriate PPE to be worn when cleaning and troubleshooting robotics

10.7 Rapid Testing (kits)

• See 3.18
● Used testing kits should be considered contaminated and disposed of appropriately in accordance with applicable local and state environmental regulations.

10.8 Molecular Testing


● Molecular testing for hematopoietic malignancies


● Unidirectional work flow and spatial separation of work areas must be strictly adhered to in addition to standard laboratory safety guidelines for open bench and instrument operations.

○ Reagent preparation is the cleanest area, then specimen preparation area, and finally product detection area.

○ Care should be taken to leave transportables e.g. pens, tape, scissors, glove boxes etc. in each designated area (see 3.19). Color coding each area and using color coded tape and color coded lab coats helps enforce spatial separations of work areas and retain transportables in their designated areas.
Change laboratory coats and gloves wash hands before entering each area (see 3.19).
10.9 Phlebotomy

● Evaluate, select, and use engineered sharps injury prevention devices that are acceptable for clinical care and provide optimal protection against injuries. Evaluation of engineered sharps injury prevention devices should include their prospective use by employees who use sharps.

● Establish a process providing annual evaluation and selection of sharps injury prevention devices

HTTP://WWW.CDC.GOV/SHARPSSAFETY/PDF/SHARPSWORKBOOK_2008.PDF

10.9.1 Sharps workbook – see 3.14

10.9.2 General recommendations

● Ensure sharps disposal containers are easily accessible in patient rooms and in patient drawing areas, never more than ¾ full, and included in the annual evaluation, selection, and use of sharps injury prevention devices.

http://www.cdc.gov/niosh/pdfs/97-111.pdf  NIOSH sharps container guide

● Ensure all applicable patient-care and infection control polices and procedures are strictly adhered to in patient drawing areas, with emphasis on:

● Wash hands with simple soap and water or antimicrobial solution to protect against external and internal exposure to blood borne pathogens. Ensure hands are washed under the following conditions:

  o before gloving
  o after gloves are removed
  o after contact with each patient or patient sample
before leaving the laboratory, drawing station, or patient room
before eating
after your hands have touched a possibly-contaminated surface

- Establish a process and procedure for specimen transport within and if applicable outside the facility.

  - If patient specimens are transported from the drawing station to another area of the facility ensure they are transported in a secondary container which has a tight fitting latchable cover and is constructed of material to contain blood spills.

    - The phlebotomy service procedure manual should include spill response and spill cleanup instructions for all areas of the hospital where there is potential for specimen spills (this includes, elevators, stairwells, etc.).

  - Ensure courier services employed by the laboratory enforce the laboratory policy for transportation of specimens and ensure documentation of personnel training and competency assessment on specimen transport including procedures for spill-response, clean up, and incident reporting.

10.9.3 Dirty vs. clean areas in the phlebotomy area and in drawing station areas

  - See 3.16

10.9.4 Pneumatic Tube Systems (PTS) – see 3.1.6

10.9.5 Personal precautions

  - See 3.2

10.9.6 Disinfection of workspace – see 3.4.1
● Good work practices: Regardless of the method, the purpose of decontamination is to protect the phlebotomist, the patient and the environment, and anyone who enters a patient room/drawing station or anyone who handles materials that have been carried into or out of the patient room/drawing station.

● Limit the phlebotomy materials brought into a patient room.

● Routinely clean environmental surfaces before setting up the patient-room/drawing station “work area” and again before leaving the patient room or after each patient in the drawing station “work area”.

● Clean any item (e.g. pen, telephone, etc.) touched with used gloves.

● Do not use alcohols or alcohol-based solutions to disinfect surface areas because they evaporate readily, which significantly decreases efficacy; instead use aqueous disinfectants such as 1/10 dilution of household bleach or the hospital-recommended disinfectant.

   ○ Use disinfectants recommended for environmental surfaces, such as 1/10 dilution of household bleach or other EPA registered disinfectants effective against HBV, HIV, and other blood borne pathogens. EPA environmental disinfectant product registration information: [www.epa.gov/oppad001/chemregindex.htm](http://www.epa.gov/oppad001/chemregindex.htm).

### 10.9.7 Disinfecting patient-room work areas and drawing stations (See 3.4.1)

● Include instructions in the procedure manual regarding what PPE to wear, how to clean, what disinfectant to use, and how to dispose of the materials. The instructions should also be posted in the phlebotomy office for reference (see 3.4).
● Allow dried blood or body fluid at least 20 minutes contact with the tuberculocidal disinfectant to allow permeation and easy removal.

● Never use a knife or other instrument to scrape dried blood or body fluid from surface areas as this can generate aerosols

10.9.8 Equipment decontamination:

● Examine equipment contaminated with blood or other potentially infectious materials prior to servicing or shipping and decontaminate as necessary. Contact the manufacturer for decontamination process.

● If decontamination of equipment or portions of such equipment is not feasible, then:
  ○ Label the equipment with a biohazard symbol and a second label applied specifically identifying which portions remain contaminated.

  ○ Convey this information to all affected employees, servicing representatives prior to handling, servicing, or shipping so that appropriate precautions will be taken.

10.9.9 Documentation training and competency assessment in phlebotomy

● Assessment should include knowledge of, and adherence to, any applicable hospital infection control policies/procedures in patient settings and the concept of Standard Precautions.
Useful resources for procedures for collecting specimens

- CLSI. Blood Collection on Filter Paper for Newborn Screening Programs; LA04-A5, Wayne, PA: CLSI 2007
- CLSI. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; MM13-A, Wayne, PA: CLSI 2005

Additional useful resources

Implementing a Needlestick and Sharps Injury Prevention Program in the Clinical Laboratory (CLSI X03RE)

HTTP://WWW.CDC.GOV/SHARPSSAFETY/PDF/SHARPSWORKBOOK_2008.PDF

SHARPS WORKBOOK: This Workbook includes several sections that describe each of the organizational steps and operational processes. A toolkit of forms and worksheets is included to help guide program development and implementation. The Workbook also contains:

- A comprehensive overview of the literature on the risks and prevention of sharps injuries in healthcare personnel;
- A description of devices with sharps injury prevention features, and factors to consider when selecting such devices; and
- Internet links to websites with relevant information on sharps injury prevention.
Transportation of specimens outside the laboratory:

US Department of Transportation publication:

11. **Blood bank**

11.1. Transfusion-transmitted diseases

11.2. Blood-borne pathogen rule

11.3. Specimen receiving and log-in/set-up station

   11.3.1. leaking containers

   11.3.2. visual contamination on outside of container

   11.3.3. loose caps

11.4. Work at the open bench

   11.4.1. Operational procedure manuals

   11.4.2. Unfixed specimens

   11.4.3. Biosafety cabinet

   11.4.4. Personal protective equipment

   11.4.5. disinfection

   11.4.6. decontamination and disposal of laboratory waste

   11.4.7. Waste management plan

   11.4.8. Employee training and competency assessment

11.5. Clean vs dirty areas of the laboratory

11.6. Instrumentation

   11.6.1. See 3.18

   11.6.2. Refrigerators and freezers

   11.6.3. Automated blood bank analyzers

   11.6.4. Total or semi-automated test systems

11.7. Test kits and reagent trays

11.8. Donor blood collection, apheresis, and disposition

   11.8.1. Processes and procedures
11 BLOOD BANK (INCLUDING DONOR COLLECTION AND COMPONENT PREPARATION)

11.1 Transfusion-Transmitted Diseases

● Many infectious agents are transmitted through transfusion of infected blood and includes hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency viruses (HIV-1/2), human T-cell lymphotropic viruses (HTLV-I/II), Cytomegalovirus (CMV), Parvovirus B19, West Nile Virus (WNV), Dengue virus, trypanosomiasis, malaria, and variant CJD. The AABB also provides information on transfusion-transmitted diseases at their website.

11.2 Bloodborne Pathogen Standard

● The Bloodborne Pathogen Standard, 29 CFR 1910.1030, is promulgated and enforced by the Occupational Safety and Health Administration (OSHA) and must be adhered to in the blood bank laboratory. It is available at:


11.3 Specimen Receiving and Log-In/Set-Up Station

11.3.1 Leaking containers

● See 3.1.1

11.3.2 Visual contamination on outside of container

● See 3.1.2
11.3.3 Loose caps

- See 3.1.3

11.4 Work at the open bench

Written procedures for blood bank should include specific work practices and work practice controls to mitigate potential exposures.

11.4.1 Operational SOPs

- See 3.1.4

11.4.2 Unfixed Specimens

- Microscopes
  - Disinfect the stage, eyepieces, knobs and any other contaminated parts after use or according to a specified schedule determined by the laboratory.
  - Select an appropriate disinfectant that will be non-corrosive to the microscope (see 3.4.1).

- Slide preparations
  - Replace glass with plastic where possible.
  - Avoid waving slides in the air or use of electric fans at an open bench to air dry slides.

  - If other instruments are used to dry slides, the procedure should have a risk/hazard assessment performed to measure aerosol risks.
• Manual cell washing – perform a risk/hazard assessment

  ○ Saline washing of cell suspensions – use of automated cell washers reduces some but not all of the hazards associated with this procedure.

  ○ Dumping saline washes into pour-off containers can generate splashes and aerosols. Splashing can be minimized by using semi automated pipettes and keeping liquid levels in pour-off containers below ¼ full. Fixed shields or appropriate water resistant PPE should be worn to protect from splashes.

  ○ When shaking small test tubes for resuspending red cell pellets and reading end-point agglutination, use fixed bench top shields or wear appropriate PPE noted above to protect from splashes and sprays.

• Perform procedures using a plasma extractor or expressor with appropriate face and eye protection to protect from sprays and possible explosion of the blood/component bags.

• Use appropriate face and eye protection when cutting donor segments during either confirmatory testing or other testing.

• Wipe outer surfaces of blood bags and components with a towel moistened with appropriate disinfectant prior to release for infusion, ensuring that the disinfectant will not compromise the plastic bag.

• If engineering controls are in place to prevent splashes or sprays at blood bank workbenches then the requirement for PPE may be modified based on assessment and evidence of the effectiveness of the engineering control to prevent exposure to splashes or sprays.
11.4.3 **Biosafety Cabinet**

- Class II biosafety cabinet (see Section 3.3) is required for all aerosol-generating processes

11.4.4 **Personal Protective Equipment**

- See 3.2.2

11.4.5 **Disinfection**

- See 3.4

11.4.6 **Decontamination and disposal of laboratory waste**

- See 3.5

11.4.7 **Waste management plan – See section 3.5**

11.5 **Clean vs dirty areas of the laboratory**

- See 3.16

11.6 **Instrumentation**

11.6.1 See 3.17

11.6.2 **Refrigerators and Freezers (also see section 3)**

All refrigerators and freezers in blood bank should have an established cleaning and maintenance protocol that will minimize contamination and extend the life of the equipment and also maintain the sophisticated cooling systems blood bank refrigerators require to provide uniform and quick temperature recovery when needed.

- Most newer blood bank laboratory refrigerators and freezers are stainless steel and have painted finishes and removable trays which make cleaning and sanitizing an easier
Manufacturer instructions for use and care of blood bank refrigerators and freezers usually include recommended PPE and type of disinfectant.

- Collaborate with the manufacturer to establish SOP’s for cleaning and maintenance if instructions are not provided with the equipment.
- Clean up blood spills immediately.
- Clean refrigerator handles and outside doors around handles at the end of each shift.
- Label the refrigerator with the universal biohazard symbol and the word, “Biohazard”

### 11.6.3 Automated Blood Bank Analyzers

Automated or semi-automated instruments are now available that are adapted either to donor collection settings or patient transfusion settings. Although these instruments have the potential to replace much of the open bench testing in blood banks and donor collection settings, use of manual testing is still being used for some antibody detection and verification procedures and in smaller laboratories. All of the blood bank automated analyzers currently approved for use in the U.S. have added features to help reduce operator exposures but have not totally eliminated potentials for exposure.

- Sample probes that move quickly or deliver fluid rapidly may generate aerosols and droplets.
- Ensure instruments are used according to manufacturer instructions.
- Ensure instrument safety shields and containment devices are in place at time of use.
- Limit the amount of hand movement near sample probes and liquid-level sensors.
• Wear gloves and use gauze pads with impermeable plastic coating on one side on instruments where the operator is required to wipe sample probes.

• Handle sample trays, sample cards and sample plates with caution and covered when not being sampled to prevent spillage.

• Fill aliquot tubes using mechanical devices and never decant (pour).

• Consider effluents of clinical analyzers as contaminated and dispose of in compliance with state and local regulations.

• Follow manufacturer instructions for routine cleaning and trouble-shooting of specimen spills on or within an instrument, including the appropriate PPE and type of cleaning solution to be used.

  o When manufacturer instructions do not include spill containment and clean up instructions:

    • Collaborate with the instrument manufacturer to develop an SOP that will effectively protect the operator and maintain and extend the instrument’s operational life.

11.6.4 Total or semi-automated test systems

• See 10.6.6

11.7 Test kits and reagent trays

• See 10.7

11.8 Donor Blood Collection, apheresis and disposition

Donor collection and pheresis areas should be considered a patent care setting and all applicable hospital patient-care and infection control polices/ procedures should be strictly adhered to.
11.8.1 Processes and procedures

Establish and maintain processes and procedures to control the quality of infectious disease testing, and safe disposition and transport of all collected blood and blood products.

- Use Standard Precautions.

- Use risk and hazard assessments to determine what/when additional PPE might be appropriate.

- Ensure cover/caps on any additional specimens are tightly secured before transport.

- Place blood or blood products that are being transported from the collection site to another location in a secondary container which, in addition to maintaining a specified temperature range, also has a tight fitting cover and is constructed of material to contain blood spills.

- Place single donor units or components issued for transfusion within a secondary container to contain spills, especially when environmental conditions might cause rupture, e.g. pneumatic tube systems.

- Select and use engineered sharps injury prevention devices that are acceptable for clinical care and provide optimal protection against injuries (see 3.14.1).

- Establish a process to provide annual evaluation of use and selection of sharps injury prevention devices. (See 3.14.1 CDC Sharps Workbook)

- Provide easy access to sharps disposal containers, never fill them more than ¾ full, and include them in the annual evaluation, selection, and use of sharps injury prevention devices (see 3.14).

- Do not store donor blood and components with patient specimens and reagent trays.
● Document training and assess competency

   ○ Include knowledge of, and adherence to, hospital infection control policies/procedures in patient settings and the concept of Standard Precautions in all documented training

● Outdated blood, blood components, and tissue should be discarded in compliance with federal, state and local regulations
12. Veterinary Diagnostic Laboratories

12.1. Introduction

12.2. Biological Risk Classification and Assessment
   12.2.1. Risk Classification
   12.2.2. Risk Assessment

12.3. General Biosafety Guidelines
   12.3.1. Hand washing
   12.3.2. Personal protective equipment
   12.3.3. Staff training
   12.3.4. Biological Spill Management
   12.3.5. Immunization

12.4. Pathology (Necropsy and Surgical Pathology)

12.5. Parasitology

12.6. Mycology

12.7. Virology

12.8. Toxicology

12.9. Hematology/Serology

12.10. Molecular Diagnostics and Rapid Tests

12.11. Storage, Packaging, and Shipping

12.12. Employer and Employee Responsibilities

12.13. Biosafety Education/Training

12.1. Introduction

The primary purpose of this section is to provide practical guidelines for work practices that minimize biosafety hazards from veterinary diagnostic specimens. Many of the biosafety practice guidelines listed in other sections of this document, focused primarily on human clinical microbiology laboratories, suffice for use in veterinary diagnostic laboratories. Similar to human clinical microbiology laboratories, the nature of the work performed in veterinary diagnostic laboratories has inherent risk to laboratory workers. According to the 2008 American Veterinary Medical Association “One Health Initiative” task force report (www.avma.org/onehealth) 60% of infectious diseases in humans are due to multi-host pathogens that move across species lines (5) and over the last 30 years 75% of the emerging human pathogen diseases (e.g. west nile virus fever, highly pathogenic avian influenza, Lyme disease) have been zoonotic (transmitted between humans and animals) (4). Thus, veterinary diagnostic laboratorians too are at risk for laboratory-acquired infections. All non-human diagnostic specimens are potentially infectious to humans, although the degree of risk is less so than for handling and examination of human diagnostic specimens. Potential infectious agents in human diagnostic specimens are by definition human pathogens. Conversely not all potential infectious agents in animal diagnostic specimens are human pathogens. The key to managing biosafety risk in veterinary diagnostic laboratories depends not only upon good general biosafety practices but most importantly on a practical risk assessment of the “unknown” diagnostic specimen.

In general, veterinary diagnostic laboratories should utilize BSL-2 practices and facilities for general veterinary diagnostic work and do practical risk assessment of incoming accessions to determine whether or not it is warranted to handle the specimens using decreased (BSL-1) or increased (BSL-3) biosafety practices or facilities. Where biosafety risk and practices differ between handling of human and animal diagnostic specimens those differences are highlighted in this section.

12.2. Biological Risk Classification and Assessment

12.2.1. Risk Classification
The tables below of risk groups and specific infectious agents are included to assist in risk assessment. Two lists of risk groups are published to facilitate the assessment of risk from different microbes and to recommend appropriate safety practices for the handling of those microbes. The World Organization for Animal Health (OIE) and World Health Organization (WHO) list four groups of biohazardous agents for humans and animals based upon level of risk and availability of effected treatment and prevention (1). The Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) guidelines propose four biosafety levels (BSLs) and recommendations for appropriate containment practices for agents known to cause laboratory acquired infections (2). The two lists of risk groups are roughly equivalent and neither makes allowance for persons who are particularly susceptible to infections by pre-existing conditions such as a compromised immune system or pregnancy. Table 1 lists the two risk group classifications and Table 2 lists the recommended practices and equipment for handling microbes within each risk group. In both risk group classification systems increasing risk levels (numbers) imply increasing occupational risk from exposure to an agent and the need for additional containment for work with that agent.

Table 1. OIE Risk Groups and CDC/NIH Biosafety level (BSL) Classifications

<table>
<thead>
<tr>
<th>OIE Risk Group</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unlikely to cause disease. Not considered infectious</td>
</tr>
<tr>
<td>2</td>
<td>Moderate individual and low community risk. Unlikely to cause serious disease or be transmitted. Effective treatment and prevention available</td>
</tr>
<tr>
<td>3</td>
<td>High individual and community risk. Causes serious infections but not readily transmitted. Effective treatment and prevention usually available</td>
</tr>
<tr>
<td>4</td>
<td>High individual and community risk. Readily transmitted and no effective treatment or prevention available</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CDC/NIH BSL Class</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well-characterized agents not known to consistently cause disease in healthy adult humans. Minimal potential hazard to laboratory personnel and the environment</td>
</tr>
</tbody>
</table>
2 Agents of moderate potential hazard to personnel and the environment
3 Indigenous and exotic agents that cause serious or potentially lethal disease as a result of exposure by the inhalation route
4 Dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease


Table 2. CDC/NIH BSL Practices and Equipment

<table>
<thead>
<tr>
<th>BSL Practices</th>
<th>Safety Equipment and Facilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Standard microbiological practices</td>
<td>None required</td>
</tr>
<tr>
<td>2 BSL1 practices</td>
<td>BSC used for specimen processing and work producing aerosols or splashes</td>
</tr>
<tr>
<td>Limited access</td>
<td>PPE (coats, gloves, face shields) as needed</td>
</tr>
<tr>
<td>Display biohazard signs</td>
<td>Autoclave available</td>
</tr>
<tr>
<td>Sharps precautions</td>
<td>Safety manual available</td>
</tr>
<tr>
<td>Staff trained with pathogens</td>
<td></td>
</tr>
<tr>
<td>3 BSL2 practices</td>
<td>BSL2 equipment/facilities</td>
</tr>
<tr>
<td>Controlled access</td>
<td>BSC used for work with all specimens and cultures</td>
</tr>
<tr>
<td>Collect baseline serum from personnel</td>
<td>PPE (gowns and masks) as needed</td>
</tr>
<tr>
<td></td>
<td>Negative pressure airflow</td>
</tr>
<tr>
<td></td>
<td>Self-closing double doors</td>
</tr>
<tr>
<td></td>
<td>Exhaust air not re-circulated</td>
</tr>
<tr>
<td>4 BSL3 Practices</td>
<td>BSL3 equipment/facilities</td>
</tr>
<tr>
<td>Clothing change before entering</td>
<td>Separate building or facility</td>
</tr>
<tr>
<td>Shower on exit</td>
<td>BSC and full body, air supplied positive pressure suite for all procedures</td>
</tr>
<tr>
<td>Decontaminate all waste on exit</td>
<td>Specialized ventilation and decontamination system</td>
</tr>
</tbody>
</table>
Generally work in routine veterinary diagnostic laboratories should assume that clinical specimens contain Group 2 agents and operate with BSL 2 practices, unless a risk assessment would indicate otherwise. On occasion veterinary diagnostic laboratories may encounter Group 3 agents and use BSL3 practices. Only under extraordinary circumstances would veterinary diagnostic specimens contain Risk Group 4 agents, which will not be included in this document. Some Risk Group 2 and Group 3 agents commonly encountered in veterinary diagnostic laboratories are listed in Table 3; the list is not meant to be all inclusive.

Table 3. Examples of some common zoonotic microorganisms in Risk Groups 2 and 3 that may be present in the veterinary diagnostic laboratory

<table>
<thead>
<tr>
<th>Group 2</th>
<th>viruses:</th>
<th>influenza viruses types A, B, C, Newcastle disease virus, Parapox virus (Orf), West Nile virus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bacteria:</strong></td>
<td><em>Alcaligenes</em> spp.; <em>Arizona</em> spp.; <em>Campylobacter</em> spp.; <em>Chlamydophila psittaci</em> (nonavian); <em>Clostridium tetani</em>; <em>Clostridium botulinum</em>; <em>Corynebacterium</em> spp.; <em>Erysipelothrix rhusiopathiae</em>; <em>Escherichia coli</em>; <em>Haemophilus</em> spp.; <em>Leptospira</em> spp.; <em>Listeria monocytogenes</em>; <em>Moraxella</em> spp.; <em>Mycobacterium avium</em>; <em>Pasteurella</em> spp.; <em>Proteus</em> spp.; <em>Pseudomonas</em> spp.; <em>Salmonella</em> spp.; <em>Staphylococcus</em> spp.; <em>Yersinia enterocolitica</em>; <em>Yersinia pseudotuberculosis</em></td>
<td></td>
</tr>
<tr>
<td><strong>fungi:</strong></td>
<td><em>Aspergillus fumigatus</em>; <em>Microsporum</em> spp.; <em>Trichophyton</em> spp.; <em>Blastomyces dermatitidis</em> (tissues); <em>Coccidioides immitis</em> (tissues); <em>Cryptococcus neoformans</em>; <em>Histoplasma capsulatum</em> (tissues); <em>Sporothrix schenckii</em></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 3</th>
<th>viruses:</th>
<th>rabies virus; equine encephalomyelitis virus (eastern, western, venezuelan), japanese encephalitis virus; louping ill virus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bacteria:</strong></td>
<td><em>Bacillus anthracis</em>; <em>Burkholderia mallei</em>; <em>Brucella</em> spp.; <em>Chlamydia psittaci</em> (avian strains only); <em>Coxiella burnetii</em>, <em>Mycobacterium bovis</em>.</td>
<td></td>
</tr>
<tr>
<td><strong>fungi:</strong></td>
<td><em>Blastomyces dermatitidis</em> spores (cultures only); <em>Coccidioides immitis</em> spores (cultures only); <em>Histoplasma capsulatum</em> spores (cultures only).</td>
<td></td>
</tr>
</tbody>
</table>
In addition to zoonotic agents, veterinary diagnostic laboratories must assess for the suspected presence of “high consequence livestock pathogens” during risk assessment. High consequence livestock pathogens are defined by the USDA Animal and Plant Health Inspection Service (APHIS) Agricultural Select Agent program in accordance with select agent and toxin regulations published in 2008 in the U.S. Federal Register by the U.S. Department of Health and Human Services (42 CFS part 73) and by USDA (9 CFS part 121 and 7 CFS part 331). Criteria used to classify high consequence livestock pathogens included severity of effect on animal products, virulence and transmissibility of the agent, and availability of effective treatment. Although not necessarily zoonotic agents, high consequence livestock pathogens can have severe detrimental economic impact on agricultural animal health and require handling using BSL-3 practices and facilities to prevent environmental dispersion and contamination.
### Table 4. High Consequence Livestock Pathogens and Select Agents

<table>
<thead>
<tr>
<th>Livestock</th>
<th>USDA/HHS Overlap Agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>African horse sickness virus</td>
<td><strong>Bacillus anthracis</strong></td>
</tr>
<tr>
<td>African swine fever virus</td>
<td><em>Brucella abortus</em></td>
</tr>
<tr>
<td>Akabane virus</td>
<td><em>Brucella melitensis</em></td>
</tr>
<tr>
<td>Avian influenza virus (highly pathogenic)</td>
<td><em>Brucella suis</em></td>
</tr>
<tr>
<td>Bluetongue virus (exotic)</td>
<td><em>Burkholderia mallei</em></td>
</tr>
<tr>
<td>Bovine spongiform encephalopathy</td>
<td><em>Burkholderia pseudomallei</em></td>
</tr>
<tr>
<td>Camel pox virus</td>
<td>Hendra virus</td>
</tr>
<tr>
<td>Classic swine fever virus</td>
<td>Nipah virus</td>
</tr>
<tr>
<td>Foot-and-mouth disease virus</td>
<td>Rift Valley fever virus</td>
</tr>
<tr>
<td>Goat pox virus</td>
<td>Venezuelan equine encephalitis virus</td>
</tr>
<tr>
<td>Japanese encephalitis virus</td>
<td>Adapted from USDA/APHIS Select Agent and Toxin List, 2010</td>
</tr>
<tr>
<td>Lumpy skin disease virus</td>
<td>(<a href="http://www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinlist">www.aphis.usda.gov/programs/ag_selectagent/ag_bioterr_toxinlist</a>)</td>
</tr>
<tr>
<td>Menangle virus</td>
<td></td>
</tr>
<tr>
<td><em>Mycoplasma capricolum</em> subspecies</td>
<td></td>
</tr>
<tr>
<td>Mycoids small colony (MmmSC) (contagious bovine pleuropneumonia)</td>
<td></td>
</tr>
<tr>
<td>Peste des petits ruminants virus</td>
<td></td>
</tr>
<tr>
<td>Rinderpest virus</td>
<td></td>
</tr>
<tr>
<td>Sheep pox virus</td>
<td></td>
</tr>
<tr>
<td>Swine vesicular disease virus</td>
<td></td>
</tr>
<tr>
<td>Vesicular stomatitis virus (exotic)-Indiana subtypes VSV-IN2, VSV-IN3</td>
<td></td>
</tr>
<tr>
<td>Virulent Newcastle disease virus</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from USDA/APHIS Select Agent and Toxin List, 2010
12.2.2. **Risk Assessment** Refer to section 2 of this document for detailed risk assessment guidelines.

- There is no official standard approach, method or one correct way to conduct a risk assessment, but there are several strategies available, such as using a risk prioritization matrix, conducting a job hazard analysis, or simply listing the potential scenarios of what could go wrong while conducting a procedure, task or activity. Risk assessment in veterinary diagnostic laboratories takes into account the likelihood of various risk group or BSL microorganisms being present within unknown clinical samples, plus the likelihood that routine processing of the clinical samples would expose laboratory workers to infectious agents within those clinical samples. A risk assessment should take into consideration the source of the clinical sample (including host species and clinical history), the suspected pathogen within a specimen with its inherent risk group characteristics, the work activity during diagnostic workup of the clinical sample in the laboratory, and the competencies and experience of the laboratory personnel.

- The most critical risk assessments for veterinary diagnostic laboratories are consideration of host species, the known medical condition and clinical history of the patient, clinical signs of the patient, and endemic local geographical conditions (many laboratories receive samples from wide and diverse geographical areas).

- Some Risk Group 3 agents are endemic to specific geographic regions or specific species and clinical syndromes and would warrant increasing BSL practices to appropriate levels. Examples include tularemia in western gray squirrels in the western USA causing sudden death, anthrax in cattle in the north central USA causing sudden death, *Coxiella burnetii* causing ovine abortions, psittacosis resulting in respiratory or enteric disease in avian birds. Alternatively, risk assessment may indicate a reduction of biosafety practices from routine BSL-2 practices. An example would be animal blood samples submitted for serology analysis. Unlike human blood samples that can harbor blood borne human pathogens such as HIV or hepatitis virus, animal serum in general does not contain zoonotic blood borne pathogens and could often, based upon a risk assessment, be handled using BSL-1 practices.
The assessment of clinical history and other data provided on a laboratory accession/submission form depends upon professional judgment and should be conducted or overseen by a qualified veterinarian familiar with the zoonotic and select agents listed in Table 3 and Table 4 above and the diseases caused by those agents. If questions arise during case accessioning and login that cannot be clarified from the accession paperwork, then the submitting veterinarian should be contacted by telephone. Knowledge regarding typical clinical signs, host range, basic epidemiology and geographical distribution of diseases caused by these agents is essential. The importance of biosafety risk assessment for veterinary diagnostic specimens cannot be overemphasized. It is the primary method to differentiate handling of diagnostic specimens in veterinary diagnostic laboratories from handling of specimens in human clinical microbiology laboratories, where the potential of diagnostic specimens containing human pathogens is much higher.

12.3. General Biosafety Guidelines

Refer to Section 3 for extensive and detailed biosafety guidelines generally applicable to all subdiscipline areas within a veterinary diagnostic laboratory. A few topics are highlighted below for emphasis in veterinary diagnostic laboratories.

The person most at risk of exposure and laboratory-acquired infection is the laboratorian working to identify a suspect infectious agent within the diagnostic specimen so the choice of laboratory work practices to prevent personal exposure is one of the most important decisions in designing a laboratory biosafety plan.

In the laboratory, the routes of exposure are limited and include inhalation of fine-droplet infectious aerosols by the airborne route, direct contact on skin or mucous membranes or ingestion of large droplet infectious material, or percutaneous transmission by needles or other sharps. The National Research Council (NRC) Committee on Hazardous Biological Substances in the Laboratory in 1989 recommended seven basic prudent biosafety practices to avoid exposure to infectious agents via the most common routes of laboratory infection.
The basic practices help prevent exposure of laboratory workers and are outlines in Table 1. The NRC recommendations were identified as the most important biosafety work practices but should be supplemented by additional practices, equipment and facility design whenever there is an increased risk of exposure to a biosafety hazard or the possibility of exposure to a BSL-3 agent.

Table 1 Prudent biosafety practices for workplaces that handle biohazardous agents

<table>
<thead>
<tr>
<th>Prudent practice / barrier protections</th>
<th>Exposure route(s) blocked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do not mouth pipette</td>
<td>A, I, C</td>
</tr>
<tr>
<td>Manipulate infectious fluids carefully to prevent aerosol production</td>
<td>A, I, C</td>
</tr>
<tr>
<td>Restrict use of sharps (needles, syringes)</td>
<td>P, A</td>
</tr>
<tr>
<td>Use protective laboratory clothing/equipment</td>
<td>C, A, I</td>
</tr>
<tr>
<td>Wash hands frequently</td>
<td>C, I</td>
</tr>
<tr>
<td>Decontaminate work surfaces</td>
<td>C, I</td>
</tr>
<tr>
<td>Do not put contaminated droplets into mouth, eyes or nose (no eating, drinking, storing foods, smoking, or applying cosmetics or contact lenses in the laboratory)</td>
<td>C, I</td>
</tr>
</tbody>
</table>


12.3.1. Hand washing

Hand washing is the most important procedure to reduce the duration of exposure to an infectious agent, and prevent dissemination of the infectious agent. Hand contamination occurs during manipulation of specimens, contact with work surfaces, telephones and equipment. Laboratory personnel should wash their hands:

- Immediately after removing gloves
- After obvious contamination
- After completion of work
- Before leaving the laboratory
• Before hand contact with nonintact skin, eyes or mucous membranes

12.3.2. Personal Protective Equipment (PPE)

● For routine work in veterinary diagnostic and clinical laboratories PPE should be provided, used and maintained in the laboratory workspace. Laboratory workers should be trained in the use of PPE for specific tasks, know the limitations of PPE and be trained in appropriate procedures for maintaining and disposing of PPE. The level of PPE use in routine veterinary diagnostic laboratory work should be sufficient for the BSL practices appropriate to the suspected or identified risk and at a minimum should include gloves and protective clothing.

● Gloves protect the wearer from exposure to potentially infectious material, should be changed frequently, and most generally are thin latex, vinyl or nitrile. Protective clothing should include fully closable long sleeved coats or gowns that extend below the level of the workbench. Open toed shoes should not be worn in the laboratory to prevent accidental spillage on bare skin. Protective clothing should not be worn outside the laboratory or taken home for cleaning or laundering.

● Face and eye protection should be used when splashes or sprays of potentially infectious material may be generated during laboratory processing, including during laboratory clean up. Face and eye protection equipment could include splash goggles, face shields or bench top splash guards.

● Respiratory protection devices (respirators) should be used as part of BSL practices appropriate to the risk assessment to prevent inhalation of potentially infectious aerosols. The decision to use respirators in the laboratory may come either from the inherent risk of potential infectious agents in a clinical specimen (e.g. BSL 3 or Risk Group 3 agents) or from laboratory manipulations necessary for agent identification that may produce infectious aerosols. The type of respiratory protection depends upon the specific hazard. If an N95 or higher-rated respirator cannot be used (i.e. because of facial hair, asthma, etc.) then a Powered Air Purifier Respirator (PAPR) should be worn when respiratory protection is required. Surgical masks are not considered a form of effective respiratory protection.
12.3.3. Staff Training

Biosafety training and education of workers about potential hazards and safe work practices are essential to creating a safe work environment. The size of the safety training program will vary with needs and could include:

- Standard Precautions (prudent biosafety practices shown in Table 1)
- Selection, use and limitations of Personal Protective Equipment
- Management of biohazardous waste
- Post exposure management, accident reporting, and investigation of incidents
- Blood-borne pathogen information
- Basic understanding of risk groups and risk assessment
- Procedures for biohazardous spills

Biosafety training should be documented (date and content of training), placed in the employee’s training record and maintained for 3 years. The effectiveness of laboratory safety training should be evaluated periodically. Safety assessments could include safety audits, inspections by outside agencies, review of accident or incident reports, and observations and suggestions made by employees. Although management provides resources to address and correct safety deficiencies, the efforts of laboratorians working at the bench top provide the foundation of a safe work environment.

12.3.4. Biological Spill Management

- The management of biological spills in clinical laboratories must account for the specific infectious agent (if known), the volume of infectious material spilled, and the presence of aerosols. Aerosols may readily transmit in spills involving BSL 3 or Risk Group 3 agents. Thus, occupants should evacuate the areas immediately, close doors and not re-enter the area for 30-60 minutes.

- When breakage occurs in a centrifuge (which inherently would produce aerosols) the equipment should remain closed for 30 minutes before decontamination commences. PPE for biological spills should include puncture resistant gloves, N-95 respirators (BSL-2 or 3) fluid impenetrable shoe covers, coats or gowns, and facial protection.
For BSL3 agents, a respirator or HEPA-filtered respirator should be used, or calling a designated, prepared spill emergency response team may be appropriate. Any broken glass in a spill area should be removed and discarded without contact with the hands (use broom, forceps, tongs etc.). A typical biological spill clean procedure involving a possible aerosol should include the following:

- Alert personnel in area and evacuate
- Close doors and do not re-enter area for 30-60 minutes (post sign forbidding entry to the area) based on the number of air exchanges.
- Alert laboratory supervisor
- Don PPE appropriate for type of spill
- Wear gown, gloves, facial protection
- Remove and discard broken glass or other objects (without contact with hands
- Absorb the spill with absorbent material
- Discard contaminated material in a biohazardous waste container
- Clean spill site with aqueous detergent
- Decontaminate area with appropriate disinfectant
- Rinse spill site with water and allow site to dry
- Copy contaminated laboratory forms and discard into the biohazard waste container
- Place all disposable contaminated clean up material in the biohazard bag and treat as infectious waste
- Wash hands
- Prepare a spill/incident report, identify cause of spill and determine remedial action

If a spill occurs in a Biosafety Cabinet do not turn off the cabinet fan. Minor spills in a BSC can be absorbed with absorbent paper. If infectious material flows into the grille, all items in the cabinet should be wiped with disinfectant and removed. The drain valve should be closed and disinfectant poured onto the surface and through the grille into the drain pan, allowed appropriate contact time then drained, rinsed and dried.

If a specimen tube breaks within the plastic screw-capped canister in a centrifuge:

- Turn the motor off
• Remove the canister immediately and place under a biological safety cabinet.
• Notify senior person in charge and other colleagues working in the area.
• While wearing protective clothing, open the canister under the safety cabinet.
• Pour a 1:10 dilution of bleach or a non-corrosive disinfectant into the canister to
decontaminate all surfaces; let the canister soak in bleach or disinfectant solution for 10
minutes. Clean canister thoroughly.
• Do not pick up broken glass with gloved hands. Use forceps or cotton held in forceps, or
tongs or hemostats and dispose into a biosafety sharps container.
• Discard all non-sharp contaminated materials from canister into a red biohazard bag for
biohazard waste disposal.
• Unbroken capped tubes should be swabbed with the same disinfectant then swabbed
again, washed with water and dried.
• All materials used during the clean-up must be treated as infectious waste.

NOTE: If the specimen tube breaks in a centrifuge that does not have
individual canisters, but does have a biohazard cover and sealed rotor, follow the
manufacturer’s instructions for cleaning and decontamination.

12.3.5. Immunization.

A current CDC guideline on immunization of health care personnel against specific
pathogens is provided in Section 14.1.4 and includes hepatitis B, influenza, measles,
mumps, rubella and varicella that are diseases whose agents are not present in animal
diagnostic specimens. In veterinary diagnostic laboratories it is not recommended that
laboratorians be immunized routinely against potential Risk Group 3 or BSL-3
pathogens. The only situation in which immunization should be considered is against
rabies virus when individuals are processing a large number of specimens potentially
containing rabies virus (e.g. routinely processing central nervous tissues from animals
with neurological disease compatible with rabies). Table 5 below lists rabies pre-
exposure prophylaxis guidelines for the United States of America according to a 2008
CDC advisory committee on rabies immunization practices.
12.4. Pathology (Necropsy and Surgical Pathology)

Refer to Section 5 for detailed biosafety guidelines applicable to necropsy, surgical pathology, and histology working areas within a veterinary diagnostic laboratory.

12.5. Parasitology

Refer to Section 6 for detailed biosafety guidelines applicable to parasitology working areas within a veterinary diagnostic laboratory.

12.6. Mycology

Refer to Section 7 for detailed biosafety guidelines applicable to mycology working areas within a veterinary diagnostic laboratory.
12.7. Virology

Refer to Section 8 for detailed biosafety guidelines applicable to virology working areas within a veterinary diagnostic laboratory.

12.8. Chemistry/Toxicology/Drug Testing

Refer to Section 9 for detailed biosafety guidelines applicable to chemistry/toxicology working areas within a veterinary diagnostic laboratory.

12.9. Hematology/Serology

Refer to Section 10 for detailed biosafety guidelines applicable to hematology and serology working areas within a veterinary diagnostic laboratory.

12.10. Molecular Diagnostics and Rapid Tests

Guidelines pertaining to biosafety best practices when conducting molecular diagnostic testing (PCR) or using rapid tests such as ELISA can be specific to the particular testing being conducted and are discussed in many of the subspecialty sections of the main document (Sections 3, 4, 5, 6, 7, 8 and 10). Section 8 (Virology) under subsection 8.10 provides the most thorough biosafety guidelines for molecular diagnostic testing.

12.11. Storage, Packaging, and Shipping

Refer to Section 13 for detailed biosafety guidelines applicable to functions within a veterinary diagnostic laboratory regarding storage, packaging and shipping of infectious or diagnostic specimens.

12.12. Biosafety Education/Training
Refer to Section 15 for practical guidelines regarding biosafety training within a veterinary diagnostic laboratory.

12.13. Biosafety Quality Improvement

Refer to Section 16 for guidelines regarding continual improvement of biosafety within a veterinary diagnostic laboratory.
References


13. Storing, packaging, and shipping infectious substances

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   13.1.1. Recommendations

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13 Storing, Packaging, and Shipping Infectious Substances

13.1 Storage of Infectious Substances

Infectious substances in a clinical microbiology laboratory are encountered as fresh and processed patient specimens, cultures and subcultures, stored isolates, and serum or plasma. Invariably, all of these substances must occasionally be stored in some form and for some length of time and many of these substances will be manipulated, relocated, and otherwise touched by laboratory workers. Therefore, storage of infectious substances is an extremely important and integral component of worker safety in clinical microbiology laboratories.

Handle all stored infectious substances using Standard Precautions and aseptic technique. Organisms responsible for external contamination of the storage vial will remain viable during storage and can be transmitted by manipulating the vial.

13.1.1 Recommendations

- Use primary containers with tight-fitting lids
- Store as far as possible from common walkways, laboratory cart, and human traffic, and reagents
- Assure restricted access to the storage site
- Assure storage at temperatures appropriate to maintain viability of microorganisms
○ Use sturdy racks, buckets, or boxes that will assure the item will remain upright.

○ Storage cabinets or refrigerators should have latching doors.

○ Use separate refrigerators to store long term cultures archives, subcultures, and processed patient specimens.

Reference


13.2 Packing and Shipping Infectious Substances

Use of reference laboratories for routine and esoteric testing has increased over time. Some of the reference laboratories may not be able to send a courier to collect the specimens from the submitting laboratory. Knowledge of specimen transportation standards and requirements needed to forward specimens using common air and ground carriers has progressively become more important for diagnostic laboratories.

The American Society for Microbiology has published and made available on-line comprehensive guidelines for packing and shipping infectious substances (1,5,7). They are based on guidelines issued by the International Air Transport Association (IATA) and the United States Department of Transportation (DOT). The information presented here is an abridged version of these guidelines and is not intended to be an all-inclusive guide.
to packing and shipping infectious substances. Permits may be needed in addition to these requirements. The information is based on IATA and DOT regulations current as of January 1, 2010.

Note: The requirements and regulations governing the transport of infectious substances change frequently. Shippers are responsible for being aware of these changes, adhering to current regulations, getting permits in advance of shipping, and interpreting applicable regulations for themselves and their facilities.

13.2.1 Governing Authorities and Regulations

- The most recognized, copied, and used packing and shipping guidelines in the world are those in the annual IATA publication Dangerous Good Regulations (8).

- In the United States, the DOT regulates the commercial transportation of dangerous goods (e.g., explosives, gases, flammable liquids, infectious substances, and radioactive materials) by both air and ground carriers (9). The DOT regulations are in substantial agreement with international standards (9).

- For practical purposes, shippers of infectious substances can consider compliance with IATA requirements to be compliance with DOT regulations.

13.2.2 Importance of Regulations

The purpose of the regulations is to protect the public, emergency responders, laboratory workers, and personnel in the transportation industry from accidental exposure to the infectious contents of the packages. An important non-safety-related benefit of adherence to these regulations and requirements is to minimize the potential for damage
to the contents of the package during transport and to reduce the exposure of the shipper to criminal and civil liability associated with the improper shipment of dangerous goods.

13.2.3 Exceptions

● The transportation of small quantities of non-Category A infectious substances (usually specimens being transported for clinical, diagnostic, or other patient care purposes) is exempt from most DOT regulations if the specimens are transported by courier, i.e. private or contract carrier in a motor vehicle used exclusively to transport such substances (9).

● Pack and secure non-Category A infectious substances inside the vehicle according to DOT regulations.

● These regulations are usually less stringent and state that the substances need only be in leak proof containers, sealed securely, and secured within the vehicle during transport. The usual strict OSHA regulations still apply during this type of transportation of infectious substances.

13.2.4 Specific Regulations

● The safe and legal transport of infectious substances is based on the following mandated activities:

   ○ Training every 2 years for individuals handling infectious substances for shipment
Classification and naming of infectious substances.

Selection of correct packaging materials.

Packing shipments correctly.

Placing appropriate markings and labels onto the outer package.

Documenting relevant aspects of each package and its contents.

13.2.5 United States Postal Service

The United States Postal Service publishes its own regulations in the USPS Domestic Mail Manual (10). The USPS regulations for mailing hazardous materials generally adhere to DOT regulations but the USPS Domestic Mail Manual should be consulted for specific needs and requirements.

13.3 Classification of Infectious Substances

13.3.1 Classification

All shipped goods must be classified using a three-step process to define dangerous goods that are shipped by commercial carriers. Classification allows the shipper to select the proper IATA packing instructions and directions to use, and provides important information necessary to complete required documentation (a Shipper’s Declaration) if the substance is a Category A infectious substance.

13.3.2 Steps of Classification

- The material is classified into one of the nine IATA-specified classes (Class 1 through Class 9) of dangerous goods. Infectious and toxic substances are Class 6 dangerous
goods; dry ice is a Class 9 dangerous good. Class 6 and Class 9 substances usually are
the only dangerous goods shipped by laboratorians.

- Class 6 substances must be divided into either Division 6.1 (toxic substances)
  or Division 6.2 (infectious substances).
- Division 6.2 infectious substances must be classified into one of nine IATA-specified
types of infectious substances: Category A infectious substance, Category B infectious
substance, Patient Specimens, Exempt Human or Animal Specimens, Genetically
Modified Organisms, Exempt Substances, Biological Products, Infected Animals, or
Medical Waste (Table 1 and Figure 1).

- If the substance is determined to be either a "Patient Specimen" or an "Organism" and
  is not obviously a Category A or Category B substance but it meets the criteria of or has
  characteristics of a Category A or Category B substance, the shipper must classify it as a
  Category A or Category B substance. Otherwise, the substance must be classified as an
  “Exempt Human or Animal Specimen” or a “Genetically Modified Organism” (Class 9),
  respectively.

### 13.3.3 Category A Infectious Substance

- A Category A substance is “an infectious substance which is transported in a
  form that, when exposure to it occurs, is capable of causing permanent disability,
or life-threatening or fatal disease to otherwise healthy humans or animals” (9).
Category A substances are specifically designated and listed by IATA and DOT. The list of Category A substances is not all-inclusive; the shipper is allowed by IATA to perform a thorough risk assessment to use their discretion and professional judgment when deciding if a substance meets Category A criteria. Category A substances must be assigned the UN number UN2814 or UN2900. See Figure 1.

**Note:** Some Category A infectious substances are considered Category A only if the substance is in culture form, i.e. concentrated; this distinction is clearly indicated in the specific IATA list.

Some Category A pathogens have been designated as agents of bioterrorism and are known as Select Agents (2,3,4,6). United States Federal regulations require shippers to have special registration and permits to possess, use, transfer, and receive Select Agents.

**13.3.4 Category B Infectious Substance**

A Category B substance is “an infectious substance that does not meet the criteria for inclusion in Category A” (9). Category B substances are not in a form generally capable of causing disability, life-threatening illness, or fatal disease. Category B substances must be assigned UN number UN3373 (Biological Substance, Category B). Examples of possible Category B substances are the following:
○ Typical clinical, diagnostic, or patient specimens, e.g., blood, biopsies, swab specimens, excreta, secreta, body fluids, tissues, etc., (a) being shipped for routine culturing or screening testing for non-Category A infectious microorganism(s) or (b) suspected of containing a non-Category A microorganism(s),

○ Typical clinical laboratory cultures (usually on solid or in liquid media) of routinely encountered non-Category A microorganisms routinely encountered and manipulated in clinical microbiology laboratories.

See Figure 1.

13.3.5 Exempt Human (or Animal) Specimens

● Exempt human or animal body site specimens are those for which there is “minimal likelihood there are pathogens present” (9). Examples of such specimens include urine or serum to be tested for glucose, cholesterol, hormone levels, prostate-specific antigen, and analytes used to evaluate heart and kidney function.

● Professional judgment and knowledge of patient medical history may used to determine if the specimen is an infectious risk or contains pathogens.
Exempt Human or Animal Specimens have less stringent packaging requirements than do Category A and Category B substances. IATA requires outer packages which contain Exempt Human or Animal Specimens to be clearly labeled as “Exempt Human Specimen” or “Exempt Animal Specimen”. DOT does not require this label on outer packages. See Figure 1.

13.3.6 Exempt Substances

Many substances commonly encountered in clinical laboratories are exempt from strict infectious substance shipping requirements. The following are examples of such substances:

- substances that do not contain infectious substances or are unlikely to cause disease in humans and animals;
- most environmental samples (food, soil, etc.);
- substances that contain neutralized or inactivated microorganisms;
- substances to be tested for alcohol or drugs, pregnancy indicators, cancer, and antibodies;
- samples submitted for forensic analysis
- dried blood spots and fecal occult blood screen specimens;
- blood and blood components collected for the purpose of transfusion or transplantation;
- FDA-approved and FDA-licensed biological products; and
- ≤30 mL of 10% formalin per primary container when the formalin is used as a preservative.
13.3.7 Patient Specimens

- A “Patient Specimen” is material collected directly from humans or animals for diagnostic, treatment, prevention, investigational, or research purposes.

- Patient specimens that have Category A or Category B criteria should be classified as Category A or Category B substances.

- Patient specimens that have neither Category A nor Category B criteria should be treated as Exempt Human or Animal Specimens. See Figure 1.

13.3.8 Genetically Modified Organisms

- Genetically modified organisms usually meet either Category A or Category B criteria. If this is not the case, the organism must be classified as a “genetically modified microorganism” (Class 9; Miscellaneous Dangerous Goods) and packed and shipped as such.

13.3.9 Biological Products

- Virtually all commercially available biological products are exempt from the regulations. Examples of biological products include bacterial typing sera,
vaccines, bacterial antigens, antimicrobial agents, reagents for identifying bacteria, and reagents used in antimicrobial susceptibility testing.

13.3.10 Infected Animal

- A live, intentionally infected animal that is known to contain or reasonably expected to contain an infectious substance cannot be transported by air unless the substance cannot be transported by any other means. An exemption from DOT will be required.

- Consultation with individual commercial carriers is advised if either live or dead infected animals need to be shipped.

13.3.11 Medical Waste

- Medical Waste that contains Category A or Category B infectious substances must be packed and shipped as such and assigned UN2814, UN2900, or UN3373.

- Medical waste that is reasonably believed to have a low probability of containing infectious substances must be packed and shipped as Medical Waste, n.o.s. (UN3291).

13.4 Naming Category A and Category B Substances
After classifying the substance, the shipper must identify (officially name) the Category A and Category B infectious substances by assigning the substance one of the 3,000+ IATA-specified and internationally recognized UN numbers and proper shipping names listed in the blue pages section of the IATA *Dangerous Goods Regulations*.

This list provides 14 informational items for each of the proper shipping names and the items correspond to the information needed to complete the Shipper’s Declaration. Fortunately, only seven of the 3,000 proper shipping names are used by most clinical microbiology laboratories:

- two for Category A substances that affect humans,
- two for Category A infectious substances that affect animals,
- one for Category B infectious substance,
- one for genetically modified organisms, and
- one for dry ice.

The blue pages provide proper shipping names, UN numbers, packing instructions, quantity limits, and other information related to packing and shipping substances.

### 13.5 Packing Instructions and Packing Substances

#### 13.5.1 Packing Instructions and Directions

IATA packing instructions (PI) describe the minimum standards for the safe transport of infectious substances.
The instructions used by clinical laboratories are those that relate to shipping Category A infectious substances (PI 602), Category B infectious substances (PI 650), and dry ice (PI 904; PI 954 after 2010.

There are no specifically numbered instructions for specimens classified as Exempt Human or Animal Specimens; however, IATA provides directions which must be followed. See Table 2 for a comparison of the details of packing instructions and directions.

13.5.2 Marking and Labeling Outer Packages

Shippers are responsible for the proper marking and labeling of the outer shipping container (commonly, a cardboard box).

The markings and labels communicate essential information regarding the shipper and consignee of the package, nature and weight of the contents of the package, the potential hazard of the substance, how the substance is packed, and information to be used in case of an emergency.

13.5.3 Specific Markings and Labels

- Shipper and Consignee – shipper’s and consignee’s name and address.
- Responsible Person -- name and telephone number of someone who can answer general questions about the shipment.
- Category A Substance – a Class 6 diamond-shaped “Infectious Substance” label, and a proper shipping name, UN number, and quantity label.
- Category B Substance -- “Biological Substance, Category B” and “UN3373” labels.
- Dry Ice -- Class 9 “Miscellaneous Dangerous Goods” and weight of dry ice label.
- Package Orientation – arrows on opposite sides of packages with >50 mL of a liquid or frozen liquid.
- Exempt Patient Specimens -- “Exempt Human Specimen” or “Exempt Animal Specimen” label. This requirement is specified only by IATA, not by DOT.
- Outer Package containing Category A infectious substance – a “UN” label; a UN inside of a circle, and a series of letters and numbers which indicate the type of package, class of goods the package is designed to carry, manufacturing date, authorizing agency, and the manufacturer.

Examples of appropriately labeled packages which contain Category A and B infectious substances are shown in Figures 2 and 3, respectively.

13.6 Documentation

13.6.1 Shipper’s Declaration.

- A Shipper’s Declaration is a legal contract between the shipper and carrier, is required to document the shipment of Category A infectious substances, must be accurate, and must be legible.
- Essentially all of the IATA-specified technical information required to complete the Nature and Quantity of Dangerous Goods section of the Declaration can be found in the blue pages of IATA Dangerous Goods Regulations.
13.6.2 Emergency Response Telephone Number.

- DOT, but not IATA, regulations state an emergency response telephone number must be provided on Shipper’s Declarations which accompany shipments of Category A infectious substance.
- The number must be monitored at all times by a person (not an answering machine, message service, pager, etc.) who has knowledge of the hazards of the material being shipped and emergency response and accident mitigation information in case a handler contacts the released contents of the package. This number also can be that of a commercial service which can provide the appropriate mitigation information. If a the phone number of a commercial service is used, then the contract number or service agreement number assigned by the commercial service needs to be entered on the document, as well as the name of the service provider.

13.6.3 Airbills

- Airbills are required to be prepared by IATA carriers to describe air cargo, and accompany shipments in transit. Some Dangerous Goods shipments, such as Biological Substances Category B shipments, require the preparation of this document, but not a Shippers Declaration. Specific preparation instructions are detailed within each IATA package instruction and in the “Documentation” section of the Dangerous Goods Regulations.
13.7 Refrigerants

Packaging must be leak proof when wet ice is used. Dry ice is a Class 9 dangerous good, it must be packaged according to PI 904 (PI954 after 2010), and its use requires completion of a Shipper’s Declaration if it is used to ship a Category A substance.

Note: Dry ice is an explosion hazard and must never be placed into a tightly sealed container! Dry ice must be placed outside the secondary container, and the outer packaging must permit the release of CO₂!

13.8 Training and Certification

Anyone involved in packing and shipping infectious substances must receive formal training in this activity; every 2 years by IATA, and every 3 years by DOT. The essential components of a training program must include the following:

- general awareness and familiarity with packing and shipping infectious substances.
- importance, nature, and contents of IATA and DOT regulations.
- hands-on and/or demonstrations of packaging and packing techniques.
- marking and labeling.
- documentation of shipments of dangerous goods.
- safety training.
- pre- and post-training testing.
- issuance of a certificate after successful completion of the training.
Acceptable training materials and methods include manuals, training courses, and workshops, all of which are commercially available from professional organizations and commercial suppliers of packaging materials for dangerous goods. A training program or workshop which includes didactic, hands-on training, and demonstrations can be developed by any hospital, laboratory, school, institution, or other facility.

IATA and DOT require all aspects of training to be documented. The most important document used to prove appropriate and timely training is a certificate which is issued after training is complete.

- Employers should keep a record for each employee who is trained.
- The record should include employee’s name, location and date of training, name of the trainer, course content, documentation of testing, and a copy of the certificate of training. IATA and DOT certification is valid for 2 and 3 years, respectively.

The DOT, through its Pipeline and Hazardous Materials Safety Administration (PHMSA), and the Federal Aviation Administration have authority to perform unannounced inspections of clinical laboratories whose employees pack and ship infectious substances, and to inspect these facilities for compliance with the training regulations, and to inspect training records at these facilities. Facilities that do not comply with prescribed regulations are subject to substantial fines.
References

   http://www.asm.org/?option=com_content&view=article&id=6342&Itemid=639

   http://emergency.cdc.gov/cotper/dsat.

3. Centers of Disease Control and Prevention. 2008. CDC Select Agent Program: Ensuring the Safe and Secure Possession, Use, and Transfer of Select Agents in the U.S.  


   American Society for Microbiology, Washington, DC.


# TABLE 1. Types and classifications of IATA Division 6.2 Infectious Substances

<table>
<thead>
<tr>
<th>Type of Infectious Substance</th>
<th>IATA Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category A Substance</td>
<td>Category A</td>
</tr>
<tr>
<td>Category B Substance</td>
<td>Category B&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Patient Specimen**

- meets Category A criteria: Category A
- meets Category B criteria: Category B
- does not meet Category A or B criteria: Exempt Human or Animal Specimen

**Exempt Human or Animal Specimen**

- Exempt Human or Animal Specimen

**Genetically Modified Microorganism**

- meets Category A criteria: Category A
- meets Category B criteria: Category B
- does not meet Category A or B criteria: Genetically Modified Organism

**Exempt Substance**

- none

**Biological Product**<sup>b</sup>

**Infected Animal**<sup>b</sup>

**Medical Waste**<sup>b</sup>

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<sup>a</sup>The proper shipping name for Category B substances is Biological Substance, Category B.

<sup>b</sup>Substance is not addressed in detail in this document.
### TABLE 2. Comparison of IATA and DOT Packing Requirements for Infectious Substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>Exempt</th>
<th>Human</th>
<th>Category B&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Category A&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Packing Requirement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inner Containers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leakproof primary (1&lt;sup&gt;o&lt;/sup&gt;) and secondary (2&lt;sup&gt;o&lt;/sup&gt;) containers</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>pressure-resistant 1&lt;sup&gt;o&lt;/sup&gt; or 2&lt;sup&gt;o&lt;/sup&gt; container</td>
<td>--&lt;sup&gt;d&lt;/sup&gt;</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>absorbent between 1&lt;sup&gt;o&lt;/sup&gt; and 2&lt;sup&gt;o&lt;/sup&gt; containers&lt;sup&gt;e&lt;/sup&gt;</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>list of contents between 2&lt;sup&gt;o&lt;/sup&gt; and outer package</td>
<td>--</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>positively sealed 1&lt;sup&gt;o&lt;/sup&gt; container</td>
<td>--</td>
<td>no</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td><strong>Outer Container</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rigid outer packaging</td>
<td>--</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>strict manufacturing specifications</td>
<td>none&lt;sup&gt;f&lt;/sup&gt;</td>
<td>few</td>
<td>many</td>
<td></td>
</tr>
<tr>
<td>name and number of responsible person</td>
<td>--</td>
<td>yes&lt;sup&gt;g&lt;/sup&gt;</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>markings and labels</td>
<td>yes&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Less&lt;sup&gt;l&lt;/sup&gt;</td>
<td>more</td>
<td></td>
</tr>
</tbody>
</table>

**Quantity Limits for Either Passenger or Cargo Aircraft**

<sup>a</sup> Specimens
<sup>b</sup> Category B
<sup>c</sup> Category A
<sup>d</sup> -- indicates not required
<sup>e</sup> Absorbent material is not required for Category B.
<sup>f</sup> None indicates no specifications.
<sup>g</sup> Yes indicates conformity with DOT regulations.
<sup>h</sup> Yes indicates conformity with DOT regulations.
<sup>i</sup> Less indicates less than the maximum allowed quantity.
<sup>j</sup> More indicates more than the maximum allowed quantity.
maximum for each 1° container  --  1 L (1 kg)  50 mL (50 g)

total maximum for outer package -- 4 L (4 kg)  50 mL (50 g)

Documentation

Shipper’s Declaration for Dangerous Goods -- no yes

emergency response telephone number -- no yes

Costs

cost of labor and materials to pack substance least more most

\[ ^a \text{packing directions for packing and shipping Exempt Human Specimens} \]

\[ ^b \text{packing instructions 650} \]

\[ ^c \text{packing instructions 602} \]

\[ ^d \text{requirement not specified by IATA or DOT} \]

\[ ^e \text{not required for solid substances such as tissue and solid agar media cultures or slant} \]

\[ ^f \text{should be “of adequate strength for its intended capacity, mass, and intended use”} \] (IATA quote)

\[ ^g \text{may be placed either on the outer package or on the air waybill} \]

\[ ^h \text{Only “Exempt Human Specimen” or “Exempt Animal Specimen” is required.} \]

\[ ^i \text{If an air waybill is used, the “Nature and Quantity of Goods” box/area must show “UN3373, Biological Substance, Category B” and the number of packages.} \]
Infectious Substance Being Shipped

**Patient Specimen** *(professional judgement required; if unable to make professional judgement, consider Category A or Category B)*

- for tests not related to an infectious disease
  or no reason to suspect the specimen is infectious
  or unlikely to cause disease in humans or animals
  or does not contain, has minimal likelihood of containing, or is not being tested for pathogens

**Patient Specimen or Other Substance** *(professional judgement required)*

- likely to contain or being tested for pathogen
  or has reasonable potential to cause disease in humans or animals

- pathogen on Cat A list and in appropriate form
  or suspected Cat A pathogen
  or being tested for Cat A
  or has characteristics of Cat A
  or cannot rule out Cat A
  or uncertain if Cat A or Cat B
  or considered a health risk to carrier personnel

**Biological Substance**

- does not contain Infectious Substance
- contains inactive or neutralized pathogens
- contains nonpathogenic organisms
- environmental sample
- dried blood spots
- forensic specimens for drug or identify testing
- fecal occult blood specimen
- decontaminated medical waste
- to be used for transplant or transfusion

**Exempt Substance**

**Exempt Human or Animal Specimen**

**Category A**

Infectious Substance

*(UN2814 or UN2900)*

**Category B**

Infectious Substance

*(UN3373)*

---

Figure 1. Practical algorithm for classifying infectious substances
FIGURE 2. A completely labeled outer package. The primary container inside the package contains a Biological Substance, Category B infectious substance and is packed according to PI 650.
FIGURE 3. A completely labeled outer package. The primary container inside the package contains a liquid Category A infectious substance and is packed according to PI 602.
14 Employer and employee involvement and responsibilities

14.1 Responsibilities of employers before an exposure

14.1.1 Exposure control plan

14.1.2 Documentation of Potential Exposures

14.1.3 Emergency response facilities

14.1.4 Immunizations

14.1.5 Education of employees

14.2 Responsibilities of employees before an exposure

14.3 Responsibilities of employers after an exposure

14.3.1 Determination of the extent of exposure

14.3.2 Documentation of exposures

14.3.3 Consultation with employee health clinicians

14.3.4 Counseling exposed employees

14.3.5 Exposure to Mycobacterium tuberculosis

14.3.6 Exposure to Neisseria meningitidis

14.3.7 Exposure to blood-borne pathogens

14.4 Responsibilities of employees after an exposure
14. Employer and Employee Involvement and Responsibilities

Introduction

The risk of obtaining a laboratory-associated infection (LAI) after physically contacting a microorganism (an “exposure”) in the workplace is real, always present, and an integral part of working in a diagnostic laboratory, and in particular the clinical microbiology laboratory. The potential for an exposure exists whenever a laboratorian manipulates and transports microorganisms, processes and stores patient specimens, and operates instruments used in the process. Diagnostic laboratories can be safe places to work if standard and appropriate safe work practices and procedures are easily accessible, understood by employees, enforced, and followed. These procedures should be properly outlined in the Exposure Control Plan and laboratory manuals. Well designed plans with the full support of the Director and higher management can reduce workers’ chances of exposures to microorganisms and can help ensure a culture of safety in diagnostic laboratories; these plans are composed of essential elements related to preventing an exposure. Another equally important element of these plans is the employer and employee involvement and responsibilities before and after an exposure. Appropriate actions taken after an exposure can greatly reduce or even eliminate the chance that an exposure will result in an LAI.

14.1 Responsibilities of Employers Before an Exposure

14.1.1 Exposure Control Plan
● Employers need to establish a formal plan outlining work practices and procedures to minimize the potential for an exposure. This plan should outline steps to take to reduce the risk of exposure.

● Employers should have a formal plan to address actions to be taken after an employee is exposed. The plan should incorporate at least the elements presented here (or the equivalent) and be easily accessible to employees.

● Both the employer and the employee should embrace the plan and rigorously participate in protecting themselves, their colleagues, the staff, and patients.

● Develop the plan prior to a first exposure with input from the director of the laboratory, laboratory workers, and from representatives of employee health and infection control or local site medical services provider to assure the inclusion of appropriate immunization, post exposure prophylaxis, and infection control measures.

● Document annual review of Safer Medical devices and other advances in research technology that can reduce or eliminate potential risks of exposure.

  ○ A Safety Culture - Develop a “Safety Culture” with appropriate buy-in from all levels of employees including upper management and front-line workers. Develop a non-punitive reporting structure for potential occupational exposures and have outlined provisions for how employees that have self-reported changes in health status will be reassigned if required without negatively influencing their job performance or review of job performance.

  ○ All potential incidents need to be reported regardless of whether the employee thinks an exposure occurred or not. The incident is evaluated by the employer and occupational physician to determine if post exposure prophylaxis is needed.
● Medical practices designated to perform these medical evaluations should be provided with the current U.S. Public Health Service recommendations for medical evaluations and post exposure prophylaxis for the infectious agents in use.

● Outreach to the medical provider is important; the medical provider needs to be prepared to evaluate clinical laboratory workers that may have had occupational exposure to a variety of infectious agents.

14.1.2 Documentation of Potential Exposures

● Develop a record or form to accommodate the following information:
  ○ date, time, and location of the exposure.
  ○ employee’s name and employee number.
  ○ names of other employees related to the incident (witnesses, etc.).
  ○ details of the incident or exposure.
  ○ brand names and manufacturer of any devices or instruments involved including lot numbers of medical devices and PPE used at the time of exposure.
  ○ relevant health information/status of the exposed employee at the time of the exposure.
  ○ immediate or remedial actions which were taken, including first aid.
  ○ actions recommended the exposed employee take immediately or the same day, (visits to employee health or the emergency department, administration of chemoprophylaxis, consultations with physicians, etc.).
  ○ results of discussions with employee health clinicians.
  ○ monitoring and follow-up plans.
  ○ space to record results of monitoring and follow-up plans.
appropriate signatures (minimum: those of the employee and the employee’s immediate supervisor).

14.1.3 Emergency Response Facilities

- Employees must receive training on the proper use of all emergency equipment.

- First aid kits must be visible and easily accessible within the laboratory. ANSI specification eye wash stations and safety showers must be readily accessible as per the requirements of the ANSI standard and any OSHA standards which mandate their placement. Cleansing skin abrasions, flushing eyes, or showering after exposures to exposed areas of the body can greatly reduce or even eliminate the chance that an exposure will result in an LAI.

- Employees should be made aware and frequently reminded of the importance and locations of these facilities. Ensure that procedures are in place to document that all equipment is functioning properly (e.g. ANSI standards for eyewashes and showers) and within acceptable expiration dates (e.g. first aid kit).

14.1.4 Immunizations

- The CDC has published recommendations for the immunization of healthcare workers against several diseases for which these workers are at high risk (1).

- The CDC strongly recommends for human health concerns all healthcare workers be immunized against hepatitis B, influenza, measles, mumps, rubella, and varicella.
● For human health, the CDC also recommends immunization of laboratory employees who work with or in certain specifically listed high-risk situations which can be encountered in laboratories, e.g., *Neisseria meningitidis* isolates from sterile sites, hepatitis A-infected primates, hepatitis A research areas, specimens which can contain wild polio virus, rabies-infected animals, and *Salmonella typhi*.

● Immunization of employees, in general, and these special situations, in particular, are most effectively addressed in institutional employee health or infection control policies. Employers should make laboratorians aware of the existence and availability of all vaccines.

● Laboratory workers who manipulate *N. meningitidis* isolates from sterile sites should make an informed decision about vaccination with quadrivalent meningococcal vaccine (serogroups A, C, Y, and W-135) (2). The vaccine will decrease but not eliminate the risk for meningococcal disease; the vaccine might be used as an adjunctive measure by microbiologists in clinical laboratories (2,3).

### 14.1.5 Education of Employees

Employees must receive initial training and refresher training at least annually and this training should be documented. Training should encompass all the necessary laboratory procedures employed in the facility that may reduce the risk of exposure. Training should include information on engineering controls in place (including safety equipment), proper procedures used in the manipulation of samples, proper personal protective equipment and procedures to be followed in the event of an exposure. Employees should be made aware and frequently reminded that (a) many reasonable and helpful actions taken after an exposure can greatly reduce or even eliminate the chance that an exposure will result in an LAI, (b) these actions are detailed in an exposure plan, (c) employee health clinicians and infection control
practitioners are available for consultation after an exposure, and (d) immunizations appropriate for workers in CM laboratories are available. They should also be made aware of symptoms of infections and follow up procedures. Document that the employee has read and understood all procedures that have been adopted in support of this formal exposure management plan.

14.2 Responsibilities of Employees Before an Exposure

● Follow Standard Precautions and other established institutional laboratory safety practices at all times.

● Never perform laboratory procedures, manipulate microorganisms, process patient specimens, or operate microbiology identification instruments if doing so will increase their risk for an LAI.

● Know what to do immediately if one suspects they have experienced an exposure, be familiar with laboratory infection control manuals and procedures and where they are located, know the location of first aid kits, eye wash stations, and showers and how to use them, and keep the supervisor informed of their health status (immunocompetency, cuts, abrasions, pregnancy, breathing problems, etc.) to determine the possibility of an increased LAI risk while performing a particular job.

○ It is a general recommendation to report changes in health status, but employees cannot be compelled to do so. There should be a provision in place on how to handle this situation.

● Know any unique signs and symptoms of any aerosol transmitted infectious agent which the laboratory isolates and manipulates on an ongoing basis and to report them to supervisors if experienced.
14.3 Responsibilities of Employers After an Exposure

14.3.1 Determination of the Extent of Exposure

- The likelihood that an exposure will result in an LAI is directly related to several variables, including:
  - concentration of the microorganism to which the employee was exposed.
  - physical form of the microorganism (broth, colony, lyophilized, aerosol, etc).
  - innate virulence of the microorganism.
  - length of time worker was exposed to the microorganism.
  - proper use of personal protective equipment.
  - immunocompetency of the exposed worker.
  - portal of entry (intact, inflamed, abraded, or cut skin; needle stick; mucous membrane; respiratory route; oral route).

- Immediately after any exposure, an employer (together with the exposed employee, if possible) should examine all of details of the exposure incident, determine the degree to which these variables (and others if applicable) were involved in the exposure, make a decision regarding the degree of exposure, and agree to an acceptable course of action to prevent or reduce the chances of the employee’s developing an LAI. Communication or consultation with the employee’s health care provider may be necessary depending upon the severity of exposure and could be essential to a successful outcome. Fortunately, most exposures in laboratories are mitigated by good hand washing. Unfortunately, some
exposures are determined to be significant enough to document and to take actions beyond hand washing.

● A root cause analysis should be initiated to determine all facts regarding the exposure, their root causes, and an action plan to correct identified causes needs to be developed, implemented, and monitored. After an incident, retraining of personnel may be in order. In addition, procedure manuals may require review to ensure appropriate safety measures are listed.

14.3.2 Documentation of Exposures

● Document on an exposure incident form all of details of the exposure and all decisions related to the exposure.

● Gather information from other personnel if they witnessed the incident or assisted in the clean-up.

● Give employees access to medical services for consultation.

● The form should be signed by the employee and the employee’s immediate supervisor.

14.3.3 Consultation with Employee Health Clinicians

● The employee and the supervisor of an employee who has experienced a potential exposure should contact the employee health physician or nurse and discuss the exposure. These clinicians are the persons most likely to provide advice regarding timely chemoprophylaxis and to able to administer appropriate antimicrobial agents.

14.3.4 Counseling Exposed Employees

● Keep the exposed employee well informed during the postexposure period as information continues to be gathered and documented.
• Tell the employee all the findings of the exposure investigation, the supervisor’s opinion regarding the extent of exposure, what and when actions are expected to happen, who he/she should see for medical consultation, where additional information can be found, which post exposure prophylaxis is advised and available.

• Counsel the employee to seek medical advice and treatment for any acute illnesses that occurs after the exposure and during follow-up. The employee also should receive a copy of the initial and final exposure report forms.

14.3.5 Exposure to *Mycobacterium tuberculosis*

• Approaches to situations in which an employee has likely been exposed to *M. tuberculosis* are best addressed in institutional employee health or infection control policies, many of which are based on CDC guidelines (1).

• If an employee is exposed to *M. tuberculosis*, e.g., possible inhalation of aerosolized *M. tuberculosis*, the employer should arrange to have the employee skin- or blood-tested to determine if an exposure has occurred. The results of this test can be compared to the employee’s baseline or previous annual test to detect a conversion.

14.3.6 Exposure to *Neisseria meningitidis*

• Employees who are exposed percutaneously to a *N. meningitidis* isolate from a sterile site should receive treatment with an appropriate antibiotic.

• Employees who have a mucosal exposure to a *N. meningitidis* isolate from a sterile site should also receive antimicrobial chemoprophylaxis.
14.3.7 Exposure to Blood-Borne Pathogens

Approaches to employee percutaneous or membrane exposures to blood-borne pathogens, e.g., human immunodeficiency virus and hepatitis viruses B and C, are most effectively addressed in institutional employee health or infection control policies, many of which are based on CDC guidelines. These policies should address the availability of post exposure prophylaxis for exposure to hepatitis B and human immunodeficiency viruses.

14.4 Responsibilities of Employees After an Exposure

- Never assume a laboratory accident or exposure is insignificant or unimportant.

- Employees must be empowered to report all incidents with the goal of protecting themselves, their colleagues, and their families without fear of reprisal. Report all exposures to the supervisor immediately and discuss the exposure to determine what, if any, actions need to be taken. Actively participate in the documentation of the exposure and provide pertinent information that will be used in the development of the corrective-action plan.

- Cooperate fully with approved post exposure processes already in place when an exposure occurs and follow prudent medical advice.

- Follow the directions of the supervisor to the degree they feel is reasonable.

References


15 **Biosafety education**

15.1 Biosafety exercises
15.2 Annual checklist of critical safety items and procedures
15.3 Regular safety mini-presentations
15.4 Monitoring compliance with safety procedures
15.5 Job aids

16 **Continuous quality improvement**
15. Biosafety Education

- The employer must develop an accurate job description so the employee understands the job responsibilities. Knowledge, skills, and abilities needed for the job are to be defined. The incoming employee should be evaluated to see if they meet these criteria. A mentoring plan should be developed and any training gaps should be filled before the employee is placed in a position that would put them at risk for exposure. The employee competency should be evaluated and documented before they are allowed to work independently.

- Educational opportunities to reinforce safe behaviors must be ongoing and supported by all levels of management and staff. In accordance with OSHA requirements, education about the risks of exposure to infectious agents should begin with a new employee’s first orientation to the laboratory or assignment to technical work and should be specific to the tasks they perform. Training must include an explanation of the use and limitations of methods that will reduce or prevent exposure to infectious materials. These include engineering controls, work practices, and personal protective equipment. Annual retraining for these employees must be provided within one year of their original training and should emphasize information on new engineering controls and practices. Annual safety training offers a chance to review key biosafety measures that may be forgotten during every day work pressures.

- It should be clear who is responsible for overseeing the safety education of the laboratory personnel. This responsibility may be delegated to the biosafety officer or other staff member who has been given additional training themselves through specialized courses or work experience and their competency to perform the training verified. Because laboratory tests may be performed outside of a traditional laboratory setting (e.g., doctor’s office, outpatient clinic,
community setting), these recommendations for training and education must be adapted to suit the employees performing tests and the individual who is overseeing them.

15.1 Biosafety exercises

Live demonstrations are most effective because they allow interaction between the laboratory worker and the expert, who can ask and answer questions. Demonstrations of common problems or exposures that laboratory scientists may encounter in their normal work duties should be presented as a learning tool for individuals or groups.

A laboratory area or work station can be rigged (or “pre-positioned”) with deliberate problems or safety errors for interactive education. What’s wrong here and how would you address it? What would you do if you encountered this problem? For example:

- Set up a BSC with excess clutter inside or demonstrate someone going in and out of the hood excessively.
- Practice what to do if you drop a liquid culture of possible *Mycobacterium tuberculosis* in the mycobacteriology lab.
- Discuss what to do if a syringe is sent to the lab with a needle attached.
- How would you handle a broken tube with a specimen in the centrifuge?
- Walk through what you would do if you got a splash of a bacterial suspension in your face/eyes/mouth.
- Demonstrate someone ejecting pipette tips where they could bounce up and strike the laboratory scientist in the face.
- How to deal with a leaking specimen in the pneumatic tube?
All proper behavior for avoiding or dealing with these demo events should be described in the standard safety practices and procedures in the laboratory safety manual.

Educational reinforcement through communications and quizzes should be ongoing and random. Unpredictable challenges may be most effective because they force quick, deliberate thought about how to respond. A specific scenario could be handed to a randomly selected technologist, while some laboratories may have the IT capacity to send safety challenges to individuals when they sign on for the day. Alternatively, it may be as simple as having a question of the week posted in the laboratory. These approaches would facilitate safety exercises on all shifts.

Safety challenges may be created as competitions between laboratory sections (chemistry vs. hematology vs. microbiology staff). Challenge each section to have the best safety record or complete the most educational events over a set time period.

The American Biological Safety Association (ABSA) has links on its website (www.absa.org/resbslinks.html) to obtain biosafety information from government agencies and other professional organizations covering rules/regulations/guidelines, biosecurity and bioterrorism, and technical links and papers, information on their week long biosafety review course that can provide continuing education for laboratory personnel.

15.2 Annual checklist of critical safety items and procedures

There is no one set of questions for an annual safety checklist. While there are common activities all personnel may perform, the list should be customized to reflect the actual job duties. Each work station should be analyzed for the type of biosafety risks associated with it, and the checklist should
target each of these risks. Try having each individual draft their own checklist for the duties they perform and have their list reviewed by their supervisor and safety officer.

15.3 Regular safety mini-presentations

Monthly safety vignettes can be presented during the regularly scheduled laboratory meetings with personnel. Employees who have had a “near-miss” or actual incident could share their experience with the rest of their co-workers in accordance with institutional privacy considerations. Having this information coming from their co-workers would increase its impact. Creative solutions to prevent biosafety problems should be elicited and discussed. Alternatively each lab employee could take a turn at presenting what they perceive as a biosafety hazard and present this at the staff meeting to keep everyone aware of daily safety issues so easily forgotten in a busy work place.

If meetings are not possible, short one-page written “safety puzzles” can be shared in the break room over coffee, or posted on the lockers, or on the lab bulletin board. Try a “what’s wrong with this picture?” format. For example:

- Show a photo of a person working outside of the biosafety cabinet with molds
- Show someone working in the biosafety cabinet with objects blocking the vent of the biosafety hood
- Show a person vortexing bacterial suspensions for antimicrobial susceptibility testing without a lid on the tube.

Post the correct answers with the next safety quiz.

Employee training and competency assessment should be documented for:

- Constructing and properly labeling containers for medical waste.
● Following all federal, state, and local regulations regarding waste management including handling, disposal, and storage of medical waste.

● Transportation of medical waste, which includes any required Department of Transportation (DOT) labeling (e.g., “known infectious substance” label) of transport containers.

● Transportation of Specimens

● Employee training and competency assessment should be documented for:
  ● Use of appropriate supplies - containers, appropriate plastic bags, labeling etc. for transport of all laboratory specimens both within and outside of the hospital facility
  ● Following all federal, state, and local regulations regarding transport of laboratory specimens.
  ● Transportation of specimens outside the facility: Useful resource

US Department of Transportation publication:

15.4 Monitoring compliance with safety procedures

To reinforce the importance of safety training and education, it should be a part of the annual performance review. Employees must participate in their annual training, follow policies, and should be active in the laboratory wide safety quizzes. Those who have a series of incidents during the evaluation period should be counseled and receive more intense re-training in those areas.
It is imperative that employees understand the importance of reporting incidents to Employee Health and their supervisor or manager. This is important for the employee’s protection to link an injury or infection to an incident that may have occurred in the workplace in order to compensate the employee appropriately and improve practices to protect others. An expedited exposure control plan should be developed whereby a laboratory employee is “streamlined” through the employee health process so that they are more likely to report an incident. The supervisor or manager should follow up on every employee who has incurred a problem to protect the employee and to make sure these incidents are monitored, and systematic risks are eliminated. Annual review of engineering controls should be performed since changes in practices and technology may put the laboratory staff at new risks. New engineering controls should be reviewed by the safety officer, risk management, purchasing, and employees who may use needles and other sharps in the course of their duties as warranted.

Laboratory sections can rotate each laboratory employees as the “safety captain” of the month to reinforce good safety behaviors and encourage their fellow co-workers to do comply with safety policies. An award can be given to the “safety captain” with the best track record for their month as determined by least infractions observed or most creative idea to prevent safety incidents.

Reserve punitive measures for egregious or repeat violations and create a positive attitude about safety, not a negative one with administrative consequences. In this regard, a laboratory could create a cadre of safety “mystery shoppers”. Periodically someone (or everyone) in the lab is assigned to be a “spy” and report on any unsafe practices that they see occurring in the lab. The purpose is not to report people, but to recognize situations they see where safe practices are not being followed.
It is not only important to monitor compliance with safety guidance but also to track and show progress on a chart the number of incidents or “near-misses” that occurred each month and applaud each month that decreases are realized.

### 15.5 Job aids

Signage of what not to do may remind personnel to comply with safety policies. OSHA, CDC, and other organizations provide job aids that can be downloaded and printed. However personnel soon become oblivious to the same sign in the same place delivering the same message. These signs should be rotated with new messages that are designed to keep staff alert to the hazards at the work stations.
16. Continuous Quality Improvement

Continuous quality improvement for biosafety should be integrated with the continuous quality improvement for the entire laboratory. Following the 12 quality system elements as defined by the Clinical Laboratory Standards Institute (CLSI), the biosafety considerations for each of the 12 elements are listed and cross-defined in the table.
Table 1. Quality System Elements (QSE), Definition, and Related Biosafety Considerations for the QSEs

<table>
<thead>
<tr>
<th>QSE #</th>
<th>QSE</th>
<th>Definition</th>
<th>Biosafety Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Organization</td>
<td>Organizational structure of responsibility is defined</td>
<td>Clear reporting structure is established for 24/7 reporting of safety incidents. Each staff member knows who to consult for biosafety reporting at all times. A chain of responsibility for overall compliance leads directly to the head of the organizational unit.</td>
</tr>
<tr>
<td>2</td>
<td>Documents and Records</td>
<td>Process and procedure documents are written and maintained</td>
<td>All incidents and “near misses“ are recorded. All safety practices and policies are written, reviewed and approved and available on all shifts to all personnel and management. Document control assures that the most current policies are in use and available. Vaccination records and safety training records are appropriately completed, maintained and easily recovered.</td>
</tr>
<tr>
<td>3</td>
<td>Facilities and Safety</td>
<td>The physical environment and space is appropriate for the</td>
<td>Facilities are designed and constructed with safety controls and minimize the risk of injury and occupational illness. Safe work practices are followed at all times.</td>
</tr>
<tr>
<td>4</td>
<td>Personnel</td>
<td>Laboratory personnel follow prescribed policies and procedures according to their job descriptions</td>
<td>Personnel have received appropriate safety training at orientation, annually, and when their duties change. Documentation of biosafety training and continuous education is maintained in their personnel file and linked to their annual review. Training requirements are part of the annual resource planning process.</td>
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</tr>
<tr>
<td>5</td>
<td>Equipment</td>
<td>Instruments and equipment used to carry out laboratory functions</td>
<td>All biohazard risks associated with operating instruments are clearly defined in standard operating procedures (SOPs). Personnel are aware of procedures to decontaminate equipment prior to maintenance or being decommissioned.</td>
</tr>
<tr>
<td>6</td>
<td>Purchasing and Inventory</td>
<td>Processes and procedure for purchasing necessary supplies and materials</td>
<td>Appropriate safety supplies (masks, gloves, gowns, biohazard disposal bags and containers) and vendors are identified and documented. Sufficient inventory of safety supplies is available so that personnel do not compromise their personal safety or the safety of others.</td>
</tr>
<tr>
<td>7</td>
<td>Process Control</td>
<td>Workflow is defined to meet customer expectations and ensure the quality of the service</td>
<td>Biohazard risks associated with operational procedures are clearly defined and referenced in SOPs. Ensure that regulatory standards are met and procedures are mapped out with quality and safety as priority goals.</td>
</tr>
<tr>
<td></td>
<td>Information Management</td>
<td>Ensure effective flow of information to comply with legal and regulatory requirements</td>
<td>Document reporting of incidents and responses back to the employee. Appropriate retention of documents to meet legal requirements.</td>
</tr>
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<td>---</td>
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<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9</td>
<td>Occurrence Management</td>
<td>Identify and analyze information which results in laboratory errors or other events</td>
<td>Document all non-compliance with established safety procedures and policies. All incidents are reported to Employee Health; all “near-misses” are recorded so that systems can be improved. A reporting mechanism exists to enable reports to be categorized to type of error so that corrective actions can be established.</td>
</tr>
<tr>
<td>10</td>
<td>Assessments</td>
<td>Assess the effectiveness of the system</td>
<td>Audits of the biosafety risks and policies should be performed annually to initiate improved methods and engineering controls.</td>
</tr>
<tr>
<td>11</td>
<td>Customer Service</td>
<td>Expectations of the customer are met or exceeded</td>
<td>The requirements for biosafety are met and personnel are satisfied with policies, work practices and engineering controls to protect them. Biological samples, reagents and other items shipped to reference laboratories (“customers”) are clearly labeled for biosafety hazards.</td>
</tr>
<tr>
<td>12</td>
<td>Process Improvement</td>
<td>Systemic review of processes identify areas for</td>
<td>Systematic review of occurrence reports as well as risks and interventions informs management planning for systematic</td>
</tr>
<tr>
<td>improvement</td>
<td>improvements.</td>
<td></td>
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</tbody>
</table>