Quest Diagnostics

TRAINING UPDATE

Lab Location:	GEC,SGAH & WAH	**Date Distributed:**	11/8/2012
Department:	Micro	**Due Date:**	12/10/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Biosafety GEC.M12, SGAH.M38, WAH.M35 v000

Description of change(s):

Understands the following changes to SOP(s):

SOP assigned new #, information from corporate sop added

Section	
4	Added the following definitions: Aerosol Transmissible Pathogens, Biological Safety Officer, Respirator, Select Agent, and Solid Front Gown.
5.2	Added Standard Microbiology Safety Practices
5.2 B	Added use of solid front gown. Updated glove instructions
5.2 C	Added Respirator Use section.
5.2 E	Expanded section on use of BSC
5.2 J	Added guidance for managing personal electronic devices in the lab
6.2 A	Added instructions to Perform all venting and sub-culturing of blood culture bottles under the BSC
6.2 A	Replaced previous blood culture venting/transfer devices with the ITL Safety Subculture device. Added additional instructions to replace gloves and wash hands.
7.2	Added section on Emergency Procedures
Addendum A	Added Laboratory Biosafety Levels Criteria
Addendum B	Added Requirements of PPE and Safety Equipment table
Addendum C	Added Aerosol Transmissible Pathogens list

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 000)

Non-technical SOP

Title	**Biosafety**
Prepared by	Ron Master Date: 09/26/2012
Owner	Ron Master Date: 09/26/2012

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Issue Date:	Local Effective Date:	

12 month (or new) management review and approval: Signature acknowledges SOP version remains in effect with NO revisions.		
Print Name	**Signature**	**Date**

Form revised 3/31/00

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1. PURPOSE

This is a clinical hospital laboratory, in which work is done with a broad spectrum of indigenous moderate risk agents that are present in the community and associated with human disease of varying severity. Biosafety level 2 practices will be followed as outlined below at SGAH and WAH.

Employees working in the laboratory are at risk of occupational exposure from the time a specimen is received in the laboratory until it is disposed. Processing clinical specimens and working with isolated pathogens poses the greatest risk. This policy has been developed to minimize the potential for occupational exposure and standardize safety practices in the microbiology laboratories of Quest Diagnostics

2. SCOPE

This SOP covers aspects of safe laboratory practice including the safe handling of infectious microorganisms.

This policy applies to all employees who work with any microbiology specimen where procedures are performed that utilize: 1) contents of the specimen or, 2) biologically produced material that results from culturing, antigen extraction or nucleic acid amplification of the specimen.

3. RESPONSIBILITY

Standard precautions must be used since all patients infected with HIV and other blood borne pathogens are not always known. Health care workers must routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluid is anticipated. The laboratory follows the guidelines set forth in the Infection Control Policy.

The CLIA named **Laboratory Director** is ultimately responsible for providing a safe environment in which employees are protected from chemical, physical, and biological hazards. See 42 CFR 493.1407(e) & 42 CFR 493.1445(e)(2).

The **Department Supervisors/Managers** are responsible for ensuring proper communication, understanding, and compliance with this procedure. This includes initiating disciplinary action if employees do not comply with the safety policies.

The **Employees** are responsible for reading and following the provisions of the procedure. They are also responsible for monitoring their co-workers and reporting any unsafe practice to the supervisor.

4. DEFINITIONS

Aerosol- mechanically produced droplets or particles suspended in air that may contain infectious organisms, often produced by centrifuging or vortexing uncapped tubes, sonicating specimens, venting blood culture bottles, heating liquids or inoculation loops too rapidly, etc.

Aerosol Transmissible Pathogens (ATPs) - a pathogen that meets one of the following criteria: (1) the pathogen appears on the list in Addendum F, (2) the Biosafety in Microbiological and Biomedical Laboratories (BMBL) recommends biosafety level 3 or above for the pathogen, (3) the biological safety officer recommends biosafety level 3 or above for the pathogen, or (4) the pathogen is a novel or unknown pathogen.

Benchtop shield- a portable or permanently mounted clear acrylic panel that is placed between the worker's face and the biohazardous material to protect the worker from splashing, spraying, or spattering.

Biological Safety Cabinet (BSC) Class II - a biological safety cabinet (BSC) is a primary engineering control designed to provide partial containment of infectious splashes and total containment of aerosols generated by many microbiological procedures. It is not to be confused with a chemical fume hood, which is not designed for biological protection.

Biological safety officer(s) - a person who is qualified by training and/or experience to evaluate hazards associated with laboratory procedures involving ATPs, who is knowledgeable about the facility biosafety plan, and who is authorized by the employer to establish and implement effective control measures for laboratory biological hazards.

BMBL- this refers to the book **"***Biosafety in Microbiological and Biomedical Laboratories",* Fifth Edition, CDC and National Institutes for Health, 2007.

Biosafety Levels (BSL) – four levels (1, 2, 3, and 4) of lab safety based on a combination of lab practices and techniques, safety equipment, and the laboratory facility. Each combination is specifically designed for the operations performed, the routes of transmission of the infectious agents encountered, and the lab function or activity. (For additional descriptions of the safety levels see Addendum A)

Engineering Controls – these are instruments or devices implemented to protect the employee by isolating or removing the hazard from the workplace. In the microbiology laboratory engineering controls may include but are not limited to biological safety cabinets

(BSC), fume hoods, safety engineered needles and syringes, benchtop shields, and sharps containers.

Personal Protective Equipment (PPE) – safety clothing or equipment worn by the employee to reduce the risk of illness or injury by the materials being processed. In the microbiology laboratory PPE may include but is not limited to a buttoned lab coat, latex or non-latex disposable gloves, and a face shield to cover eyes, nose, and mouth. General work clothes (e.g. scrubs, uniforms, street clothes) are not considered to be personal protective equipment.

Respirator – a NIOSH approved device worn over the nose and mouth to prevent inhalation of harmful substances. For microbiology this equates to a N95 or higher respirator. NOTE: employees using a respirator must participate in a respirator program that includes a medical evaluation and annual fit testing.

Safety manual – this will refer to the Quest Diagnostics Environmental Health and Safety Manual.

Select agent – any of a number of agents or toxins with the potential to pose a sever threat to public health and safety. See the link below for the current list of these agents:
http://www.selectagents.gov/Select%20Agents%20and%20Toxins%20List.html

Sharps – any item that can penetrate the skin including, but not limited to, needles, scalpels, glass pipettes, broken tubes, slides and coverslips.

Sharps container – a container that is closable, puncture resistant, leakproof on sides and bottom, and labeled in fluorescent orange or orange-red, and bearing the Biohazard legend.

Solid front gown – a long sleeved fluid resistant wrap-around gown that secures in the back providing an uninterrupted barrier for the front of the employee. The gown should have knit cuffs on the sleeves that go under protective gloves to maintain the complete barrier.

Stored Isolates – this term applies to any organism kept in storage for QC or other purposes such as for scientific studies.

5. PROCEDURE

5.1 General Standards

The following standards and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Levels 2.

Safety Equipment (Primary Barriers):

- The biohazard safety cabinet will be utilized for processing/plating all specimens.
- Gloves are to be worn while performing any microbiology bench work including the plating of all specimens including blood cultures and reading and handling of all inoculated culture plates and tubes.

- Lab coats are supplied by Quest Diagnostics.
- Masks, although not necessary, are available.

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when work is in progress.

2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.

3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.

5. Policies for the safe handling of sharps are instituted. Never recap needles; dispose of them after use in proper sharps containers. Use only needle locking syringes, syringes with permanently attached needles or syringes that re-sheathe the needle

6. All procedures are performed carefully to minimize the creation of splashes or aerosols. With good microbiological techniques, moderate-risk agents can be used safely on the open bench.

7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.

8. All cultures, stocks, and other regulated wastes are disposed of via biohazard waste management system.

9. Broken glassware must not be handled directly by hand but must be picked up with a mechanical device such as a brush and dustpan, tongs or forceps.

10. Familiarize yourself with the Chemical Hygiene Plan and Material Safety Data Sheets (MSDS'S) for chemicals you will be using during testing.

B. Special Practices

1. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.

2. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).

3. Biosafety procedures are incorporated into standard operating procedures or in a safety manual adopted or prepared specifically for the laboratory. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

4. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.

5. A high degree of precaution must always be taken with any contaminated sharp items, including needles, syringes and slides.

Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as phlebotomy. Plastic should be substituted for glass whenever possible.

6. Biohazard bags are provided for the disposal of all infectious waste Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping. Place sharp items in a Sharps container before placing in the biohazard bags.

7. Laboratory equipment and work surfaces must be decontaminated with effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to local, state, or federal regulations before it is sent for repair, maintenance or packaged for transport or removal from the facility.

8. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the Laboratory Director and Safety Officer. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets (BSC) and other appropriate personal protective equipment or physical containment devices are used whenever:

Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, opening containers of infectious materials whose internal pressures may be different from ambient pressures.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.

3. Protective laboratory coats designated for laboratory use are worn while in the laboratory.

   This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory and should never be taken home.

4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces and they should not be worn outside the lab. Alternatives to powdered latex gloves are available. Hands are washed following removal of gloves.

D. Laboratory Facilities (Second Barriers)

The laboratory maintains several sinks for hand washing.

The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.

Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Biological safety cabinets are installed in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Biological safety cabinets are located away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.

An eyewash station is readily available.

Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.

5.2 Standard Microbiology Safety Practices

Specimen processing and work-up of cultures ordered for routine microbiology tests can be performed in a Biosafety Level 2 (BSL2) environment. Safety practices for a BSL2 laboratory include the following:

A. Personnel

Personnel working in the laboratory should be limited to those who:

- have received safety training (New Hire Orientation and department specific training),
- have read the safety manual,
- have read and demonstrated understanding of departmental procedures and have documented training and competency assessments on file,
- have received (or declined) appropriate immunizations or tests for agents potentially present in the lab (Hepatitis B vaccine, Meningococcal vaccine, and TB skin test).

B. Personal Protective Equipment (PPE)

- Wear a fully buttoned or zippered lab coat at all times while in the laboratory. Lab coats with knit cuffs on the sleeve to tuck under gloves are recommended.
- Wear a solid front fluid-resistant gown when handling mycobacterial specimens/cultures, high virulence virology samples/cultures (e.g. SARS), or any growing culture known or suspected of containing a select agent or highly virulent organism. (e.g. LPS survey).
- Wear latex or non-latex disposable gloves anytime there is a potential for contamination from specimens or suspensions of organisms. Stretch glove over knit cuff of labcoat or gown (if present) for optimum protection. Bandage open cuts and scratches on hands prior to wearing gloves. Replace gloves immediately if they become torn, punctured, or visibly contaminated. Wash hands after removing gloves.
- Use face shield and nitrile or other chemically resistant gloves when handling hazardous chemicals or reagents.
- Use face shields, surgical masks with eye protection or fixed safety benchtop shields when a potential for splashing, spraying, or spattering exists.
- Remove all PPE when leaving the lab area for any reason including fire drills. Never go to the cafeteria, vending area, rest rooms, lounge, or leave the building while wearing PPE.

C. Respirator Use

- Respiratory protection may be required where engineering controls (e.g. certified BSC), safe work practices and PPE listed in this SOP do not fully mitigate the aerosol exposure risk to workers. Any respirator used must be a NIOSH approved device with a rating of N95 or greater. Use of a respirator requires employee participation in a respirator program that includes a medical evaluation and fit testing as specified by OSHA (29 CFR 1910.134).

D. Sharps Safety

- Handle all sharps carefully and discard in designated sharps containers.
- Specimens sent to the lab in syringes or containers with the needles still attached pose an additional risk. Process irretrievable specimens with management approval. **Allow only experienced technical staff exercising extreme care to handle these samples if they must be processed.** Dispense sample into a Port-a-cul tube/vial, or other similar holding media appropriate for the culture ordered, until plated. Discard the needle and syringe into a sharps container. Local business unit policies should indicate the process for notifying the client and documenting the issue to prevent reoccurrence.
- A syringe with attached needle should not be used in any lab process unless there is no other alternative. Safer options must be used whenever possible. If a needle must be used, ensure it is a safety needle (1mL syringe w/ Safety Lok™ 25g, 5/8 needle, SC#34277) and is handled by staff trained in its use.
- Used disposable needles must never be bent, sheared, broken, recapped, removed from the syringe, or otherwise manipulated prior to being placed in a sharps container.
- Specimens are occasionally received on a scalpel blade. Only experienced technical staff exercising extreme care will handle these specimens. Using forceps if necessary, carefully scrape the specimen from the blade for processing. Discard the blade in a sharps container.
- Do not use a scalpel blade without a handle when a scalpel is required for processing tissue or nail samples. Always purchase "safety scalpels" that have a blade cover or sheath to prevent accidental lacerations.
- Broken glass must be placed in a sharps container or "Broken Glass" box using forceps or a mechanical device and decontaminated prior to disposal, if necessary.
- Sharps containers must be monitored closely and not allowed to overfill. Never push needles/syringes or place hands into a partially filled sharps container. Discard closed sharps containers in the biohazard waste container.

E. Class II Biological Safety Cabinet (BSC) Use

- Use a BSC anytime there is a potential for aerosol production. A BSC will also offer better protection than a face shield or bench top shield when there is a potential for splashing, spraying, or spattering.
- Subculture all blood culture bottles under a BSC and screen all plates under the BSC until high-risk pathogens have been ruled out. Secure plate lids (e.g. with parafilm or tape) to prevent accidental opening when gram stain morphology indicates the potential for a high-risk pathogen.
- Locate BSCs away from disruptions to air currents including supply and return air vents for heating/cooling, high traffic areas, open windows, or doors that are repeatedly opened.
- Decontaminate the working surface of the cabinet before the start of tasks and when work is completed. For a list of applicable disinfectants see section F below. Document completion of this task on a log sheet.
- Do not block the airflow vents at the front and rear of the cabinet with equipment and working materials. Keep minimal equipment and materials inside the cabinet to allow for proper airflow.

- Provide a method of indicating proper airflow within the cabinet. This may be a vane anemometer, paddle-wheel flow meter, piece of ribbon, or an electronic velometer. Check and document proper airflow daily or prior to each use of the cabinet.
- Keep all operations at least four inches away from the front grill on the work surface.
- Keep arm movements into and out of the cabinet to a minimum to reduce the disruption of the airflow.
- Keep a small sharps container and biohazard waste container in the BSC work area for easy disposal of sharp objects and small contaminated items.
- Do not use biological safety cabinets for protection from chemical odors or vapors unless they are designed with spark-proof electrical components and external exhaust (Class II type B).
- Certify the performance of biological safety cabinets at least annually using an authorized vendor or qualified Biomedical Engineer.
- The maintenance vendor must decontaminate the biological safety cabinet including the HEPA filter prior to moving, maintenance, or disposal. Use of formaldehyde gas or another appropriate disinfectant following the manufacturer's instructions is recommended. Re-certify any hood that has been moved before use.

F. Centrifugation

- Ensure that centrifuge tubes are intact and properly balanced prior to centrifuging.
- Centrifuge tubes and vials in sealed safety containers. Apply adhesive sealer to microtiter plate prior to centrifuging. Use sealed safety caps or covers when working with mycobacterial specimens, virology samples for spin amplification using shell vial or plate technology, and any specimen known or suspected of containing ATPs, a select agent or highly virulent organism. (e.g. LPS survey). Safety caps are also recommended for centrifuging other specimens. Do not open the safety caps to remove tubes until they are placed inside of the BSC. Regularly examine safety cap o-rings to confirm that they provide an adequate seal, and replace when necessary.
- Do not operate a centrifuge under a biological safety cabinet because air turbulence can allow aerosols to escape the cabinet.
- Clean up any tube breakage or spills in the centrifuge with an appropriate disinfectant following the manufacturer's instructions or local procedures.
- Clean the centrifuge and its components periodically with an appropriate disinfectant cleaner following manufacturer's directions for dilution and contact time.

G. Facility and Equipment

- Use electric incinerators to sterilize metal loops and needles. Cool loops and needles in a fashion that will avoid aerosolizing organisms and searing the surface of media. Alternately, disposable plastic loops and needles can be used. Do not use Bunsen burners or open flames in the laboratory.
- Decontaminate work surfaces after each shift and anytime there is a splash or spill of potentially contaminated material. Document completion of this task

on a log sheet. **Read the label on the commercial disinfectants and make sure it is designed for the agents present**. **Follow the instructions regarding dilution, contact time and material being disinfected.** Appropriate disinfectants include:

- Bacteriology – 10% bleach solution, 70% ethyl or isopropyl alcohol or a quaternary ammonium compound.
- Virology – 10% bleach solution or phenolic derivatives.

Note: For maximum effectiveness, prepare 10% bleach solution fresh each day.

- Designate and label items in the lab (phones, keyboards, calculators, staplers, etc.) as "contaminated" or "clean" based on the typical use of the item. Wear gloves during use if items are designated as "contaminated". Remove gloves prior to use if designated as "clean".
- Place a biohazard warning sign on the entrance to the laboratory and the type of PPE required for entry.
- Biological spill and chemical spill clean-up instructions should be posted in appropriate areas such as places where chemicals are used or where microorganisms are handled. Ensure they are readily visible to testing personnel. Spill kit material should be sufficient to contain a spill of the largest single container.
- Locate hand washing sinks and an eyewash station in or near the laboratory that processes microbiology specimens. Ensure eyewash nozzle caps are in place.
- Only trained employees should operate an autoclave. Use heat resistant gloves and follow the operating procedure to reduce the risk of injury.
- Only employees trained on the hazards and proper handling of compressed gas cylinders should be involved in changing cylinders.

H. Biohazardous Waste Handling

- Decontaminate all other cultures, stocks, and contaminated waste on-site prior to disposal or place in durable, leakproof containers that are closed when not in use prior to being transported from the department for disposal by an outside medical waste vendor.
- Train maintenance or housekeeping personnel on the safe handling and transportation of containers to the waste storage area, if applicable.
- See the "*Biohazardous Waste Management*" procedure in the safety manual for more information.

I. Miscellaneous Safety Practices

- Perform all procedures carefully to avoid splashing and aerosol production (e.g. vortexing, vigorous mixing or shaking, grinding, centrifuging). Keep tubes capped or sealed to reduce the risks.
- Employees must wash their hands after working with biohazardous material, after removing gloves, and before leaving the laboratory.

- Eating, drinking, smoking, chewing gum, handling contact lenses, and applying cosmetics are prohibited in the laboratory. Store food and drink outside the lab area in cabinets or refrigerators designated for that purpose.
- Use extreme care to avoid hand contact with the eyes, nose and mouth while working in the microbiology lab.
- Mouth pipetting is prohibited. Always use mechanical devices.
- "Sniffing" culture plates is prohibited as this may increase the risk of occupational exposure to infectious agents.
- Keep quantities of flammable liquids on the benchtop to a minimum. Store stock supplies of stains, alcohols, acetone, etc. in a flammable liquid cabinet. See *Flammable Liquid Storage Policy* in the national EH&S safety manual.

J. Managing Personal Electronic Devices in the Lab

Use of personal electronic device (cell phones, MP3 players, etc) in the lab by employees and vendor service technicians must comply with local Department of Health regulations and business unit policies. The following guidelines must be followed to prevent contamination of the device, which in turn may contaminate the user, thereby presenting a health risk.

- Any device carried by a lab tech working with specimens must be kept under the lab coat (e.g. in clothing pocket). Any accessory cords (e.g. to ear phones) must also be run under the lab coat.
- The device/accessories must not be touched while wearing PPE or while performing tasks that may result in contamination of the device. Remove PPE (gloves, lab coat) and wash hands prior to handling the device.

K. Ergonomic Considerations

- Ensure the chair is adjusted correctly to provide support to the back and legs while performing long sessions of specimen plating, culture reading, or data entry. This will reduce muscle fatigue.
- Extended time using the microscope can lead to stress and strain on the body. Follow these steps to remain comfortable.
  - Adjust the chair so that elbows and knees are at an approximate 90^{o} angle.
  - Adjust the back of the seat so that it provides support to the lower back area.
  - Sit up straight and pull the microscope close to avoid leaning forward.
  - Use microscopes with adjustable heads so that the oculars are at the correct height and width.
  - Take frequent stretch breaks during long periods of reading to reduce muscle fatigue.
  - Look away from the microscope and refocus on a distant object occasionally to reduce eyestrain.

6. SPECIMEN PROCESSING/SET UP:

6.1 General Information

A. All respiratory samples, regardless of test requested, will be set-up under the biohazard hood to avoid possible transmission *of Mycobacterium tuberculosis* or other respiratory pathogens.

B. When a routine culture is ordered along with an AFB or fungus culture, set up the routine culture under the biohazard hood.

C. Gloves and disposable lab coat must be worn at all times while processing specimen under the biohazard hood.

D. Use gloves when handling any specimens containing blood or blood products. Use gloves when plating urines or stools.

E. Use eye and face protection (face shield, bench shield) when performing any procedure that might produce splashes such as pouring liquid specimens.

6.2 Routine Bacterial Cultures

A. Culture Reading

1. Blood cultures

- **<u>Perform all venting and sub-culturing of blood culture bottles under the BSC.</u>**
- The ITL Safety Subculture device is the only approved method for venting/subculturing blood culture bottles (see Addendum C for pictures and ordering information)
  NOTE: Syringes with needles (safety needle or conventional needles) are prohibited from use to subculture blood cultures.
- Handle all subcultures from blood cultures under the BSC until uncommon virulent pathogens have been ruled out.
- Keep a sharps container within reach in the BSC to avoid excess movement of the contaminated venting device.
- Allow Gram stain slides to dry, and alcohol or heat fix, before removing from the BSC.
- Wear lab coat and gloves at all times while working with the BSC. Replace glove regularly, or if torn, punctured, or visibly contaminated. Wash hands after removing gloves.
- Use a bottle rack or cart to transport bottles to and from the BSC.

6.3 Virology

A. Specimen Processing, Culture Maintenance and Virus Identification

- Perform all specimen processing, inoculating, slide preparation and any other process or procedure for which the specimen container or inoculated culture is opened in a BSC.
- Wear buttoned or zipped lab coats and gloves while working in the BSC.

- Use of plastic disposable single-use sterile pipettes is preferred to glass Pasteur pipettes. Safely contain all waste materials, used pipets and glassware, and other used materials. Properly sterilized or disinfected all material according to local business unit policies or regulations before removal from the facility for disposal.

7. DISINFECTION OF ACCIDENTAL SPILLS:

7.1 General Information

A. If a spill occurs involving a patient's specimen or breakage of culture tubes, etc., pour 10% bleach over the contaminated area, cover with paper towels and let stand at least 15 minutes. Using paper towels and wearing gloves, discard the contaminated material in a biohazard trash bag.

B. If a tube breaks in the centrifuge, wait 10 minutes for dispersion of the bioaerosol, carefully remove the spilled and broken material wearing gloves. Clean the centrifuge with paper towels and 10% bleach. Autoclave the centrifuge bucket.

7.2 Emergency Procedures

Employees using proper microbiology techniques and following the procedures outlined in this document will significantly reduce the risk of exposures and injuries. However, if an incident does occur consult the supervisor and follow the guidelines below. Complete an *Incident and Injury Investigation Report* and inform the local EHS Manager or Specialist.

If this happens:	Then do this:
Microbiological contamination on lab coat or street clothes.	Remove contaminated clothing and wash thoroughly with soap and water, or decontaminate with an appropriate disinfectant solution. If contamination soaks through to the skin follow the next step.
Microbiological contamination on intact skin	Wash thoroughly with soap and water.
Microbiological contamination in an open wound or on chapped or abraded skin.	Wash thoroughly with soap and water, consult the company designated medical provider.
Microbiological contamination in mucus membranes.	Rinse with water for 5 minutes and consult the local EHS resource or medical provider if no EHS resource is available.
Blood or other potentially infectious material (OPIM) contamination on lab coat or street clothes.	Remove contaminated clothing and decontaminate by soaking in an appropriate disinfectant solution. If contamination soaks through to the skin follow the next step.
Blood or OPIM contamination on intact skin.	Wash thoroughly with soap and water.
Blood or OPIM contamination	Wash thoroughly with soap and water, immediately

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If this happens:	Then do this:
in open wound or on chapped or abraded skin.	follow the *Post Exposure Management Policy* found in the safety manual.
Blood or OPIM contamination in mucus membranes.	Rinse with water for 5 minutes and immediately follow the *Post Exposure Management Policy* found in the safety manual.
Blood or OPIM contamination on benchtop or floor.	Remove any glass or plastic with a mechanical device (e.g. forceps, cardboard or scoop) and discard in sharps container. Reduce the organic matter by removing or absorbing most of the sample with paper towel or cardboard. Cover the spill area with paper towels, soak the area with an appropriate EPA approved disinfectant and let sit for recommended contact time of the manufacturer. Discard all cleaning material in the biohazardous waste. Clean the area again with disinfectant and allow to air dry. **Note**: 10% bleach may not be optimal as it is inactivated by substances with high protein content.
Chemical contamination on the clothes or skin.	Remove affected clothing and wash skin with soap and water for 15 minutes. If chemical is caustic, contact the local emergency response personnel or the company designated medical provider immediately.
Chemical contamination of eyes or mucus membranes.	Wash affected area with water for 15 minutes. If the chemical is hazardous, contact the local emergency response personnel or the company designated medical provider immediately.
A growing bacterial culture plate or broth is dropped and broken or spilled.	Carefully remove the glass or plastic with a mechanical device (e.g. forceps or scoop) and discard in sharps container. Reduce the organic matter by removing or absorbing most of the sample with paper towel or cardboard. Cover the area with paper towels, soak the area with an appropriate disinfectant and let sit for 15 mins. Discard all cleaning material in the biohazardous waste. Clean the area again with disinfectant and allow to air dry.
Spill of Un-Processed TB or fungal culture specimen in a general laboratory area	Isolate the area directly around the spill and alert workers to stay out of the area to prevent a slip incident and further spread of the spilled material. Put on appropriate PPE including but not limited to labcoat, gloves and face shield. Cover the spill with paper towels and saturate with an EPA approved tuberculocidal or fungicidal disinfectant (depending on type of spill). Allow sufficient contact time for the disinfectant to work as defined by manufacturer (see label instructions).

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If this happens:	Then do this:
	Scoop up spill and absorbent material using mechanical devices (e.g. scoop) if contents contain broken glass or other sharps. Do Not Use Your Hands. Wipe area with additional disinfectant, as needed, and allow to air dry. Discard all spill clean-up material into the biohazard waste container. Notify the laboratory manager and EHS Manager or Specialist. Complete the Incident and Injury Investigation Report

8. SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS

Biosafety Level	Practice & Techniques	Safety Equipment	Facilities
1	Standard microbiological practices	None; primary containment equipment provided by adherence to standard laboratory practices during open bench operations	Basic
2	Level 1 practices plus: laboratory coats; decontamination of all infectious wastes: limited access protective gloves and biohazard warning signs as indicated	Partial containment equipment (i.e., Class I or II Biological Safety Cabinets) used to conduct mechanical and manipulative procedures that have high aerosol potential which may increase the risk of exposure to personnel	Basic
3	Level 2 practice plus: special laboratory clothing; controlled access	Partial containment equipment used for all manipulation of infectious material	Containment
4	Level 3 practices plus: entrance through change room where street clothing is removed and laboratory clothing is put on; shower on exit; all wastes are decontaminated on exit from facility	Maximum containment equipment (i.e., Class III Biological Safety Cabinet or partial containment equipment in combination with full-body, air-supplied, positive-pressure personnel suit) used for all procedures and activities	Maximum Containment

A. Biological agent classifications - Recommended Precautions

1. Parasitic agents
    a. Nematodes of humans (Strongyloides and Ascaris) - Biosafety Level 2
    b. Protozoal parasites - Biosafety Level 2
    c. Tramatode parasites - Biosafety Level 2
    d. Cestode parasites - Biosafety Level 2

2. Fungal agents
    a. *Blastomyces dermatitidis* - Biosafety Level 2 practices for activities with clinical materials and biosafety Level 3 practices for processing mold cultures, soil, or other environmental materials.

b. *Coccidioides immitis* - same as 2.a. above
c. *Cryptococcus neoformans* - Biosafety Level 2
d. *Histoplasma capsulatum* - same as 2.a. above
e. *Sporothrix schenckii* - Biosafety Level 2
f. Pathogenic members of the genera *Epidermophyton, Microsporum*, *Trichophyton, Penicillium marneffei, Exophiala (Wangella) dermatitidis, Fonsecaea pedrosoi, Ochroconis gallopavum, Clauduphialopora bantians* and *Ramichlorisium* - Biosafety Level 2

3. Bacterial agents
a. *Bacillus anthracis*. Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities using clinical materials and diagnostic quantities of infectious cultures. Biosafety Level 3 practices and facilities are recommended for work involving production volumes or concentrations of cultures and for activities which have a high potential for aerosol production.
b. *Bordetella pertussis.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical materials or cultures. Animal Biosafety Level 2 should be used for the housing of infected animals. Primary containment devices and equipment (e.g., biological safety cabinets, centrifuge safety cups, or specially designed safety centrifuges) should be used for activities likely to generate potentially infectious aerosols. Biosafety Level 3 practices, procedures, and facilities are appropriate when engaged in large scale production operations.
c. *Brucella* spp. Biosafety Level 2 practices are recommended for activities with clinical materials of human or animal origin containing pathogenic *Brucella* spp. Biosafety Level 3 practices, containment equipment, and facilities are recommended, respectively, for all manipulations of cultures of the pathogenic *Brucella* spp.
d. *Chlamydia psittaci* and *C. trachomatis* Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities involving the necropsy of infected birds and the diagnostic examination of tissues of cultures known or potentially infected with *Chlamydia psittaci* and *C. trachomatis.*
Biosafety Level 3, may be indicated for activities with high potential for droplet or aerosol-production and for activities involving production quantities or concentrations of infectious materials.
e. *Clostridium botulinum.* Recommended precautions.
Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities with materials known or potentially containing the toxin.
Biosafety Level 3, may be indicated for activities with a high potential for aerosol or droplet production, those involving production quantities of toxin, and those involving purified toxins.
f. *Clostridium tetani.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities involving the manipulation of cultures or toxin.
g. *Escherichia coli* (0157). Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials or cultures. Animal Biosafety Level 2 facilities and practices are recommended for activities with experimentally or naturally infected animals.
h. *Francisella tularensis.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with c linical materials containing *F. tularensis*. Biosafety Level 3 practices and facilities are recommended, respectively, for all manipulations of cultures and for experimental animal studies.
i. *Helicobacter pylori.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures known to contain or potentially containing the agents. Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with experimentally or naturally infected animals.
j. *Leptospira interrogans* - all serovars. Recommended precautions. Biosafety 2 practices, containment equipment, and facilities are recommended for all activities involving the use of manipulation of known or potentially infectious tissues, body fluids, and cultures and for the housing of infected animals. Gloves are recommended for the handling and necropsy of

infected animals and when there is the likelihood of direct skin contact with infectious materials.

k. *Legionella* spp. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of known or potentially infectious clinical materials or culture and for the housing of infected animals. Primary containment devices and equipment (e.g., biological safety cabinets, centrifuge safety cups) should be used for activities likely to generate potentially infectious aerosols.

l. *Listeria monocytogenes.* Recommended Precautions: Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical specimens and cultures known or suspected to contain the agent. Gloves and eye protection should be worn while handling infected cultures.
Animal Biosafety Level 2 practices, containment equipment and facilities are recommended for activities with experimentally or naturally infected animals. Pregnant women who work with *Listeria monocytogenes* in the clinical or research setting should be fully informed of the potential hazards associated with the organism, including potential risks to the fetus.

m. *Mycobacterium leprae.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities with known or potentially infectious clinical materials from infected humans and animals. Extraordinary care should be taken to avoid accidental inoculation with contaminated sharp instruments.

n. *Mycobacterium* spp. other than *M. tuberculosis, M. bovis,* or *M.leprae.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials and cultures of *Mycobacterium* spp. other than *M. tuberculosis* or *M. bovis.* Animal Biosafety Level 2 practices, containment equipment, and facilities are recommended for animal studies with mycobacteria other than *M. tuberculosis, M. bovis* or *M. leprae*.

o. *Mycobacterium tuberculosis, M. bovis.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for preparing acid-fast smears and for culturing sputa or other clinical specimens, provided that aerosol-generating manipulations of such specimens are conducted in a Class I or II biological safety cabinet.
Biosafety Level 3 practices, containment equipment, and facilities are recommended foractivities involving the propagation and manipulation of cultures of *M. tuberculosis* or *M. bovis.*

p. *Neisseria gonorrhoeae*. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of clinical materials or cultures. Gloves should be worn when handling infected laboratory animals and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for aerosol or droplet production and the activities involving production quantities or concentrations of infectious materials

q. *Neisseria meningitidis*. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with high potential for droplet or aerosol production and for activities involving production quantities or concentrations of infectious materials.

r. *Pseudomonas pseudomallei.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious body fluids and tissues. Gloves should be worn when handling, and during necropsy of infected animals and when there is the likelihood of direct skin contact with infectious materials. Additional primary containment and personnel precautions, such as those described for Biosafety Level 3, may be indicated for activities with a high potential for aerosol or droplet production and the activities involving production quantities or concentrations of infectious materials.

s. *Salmonella cholerasuis, S. enteritidis* (all serotypes). Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with clinical materials known or potentially containing the agents.

t. *Salmonella typhi.* Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials and cultures.

u. *Shigella* spp. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities utilizing known or potentially infectious clinical materials and cultures.

v. *Treponema pallidum*. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the use or manipulation of blood or lesion materials from humans or infected rabbits. Gloves should be worn when there is a likelihood of direct skin contact with lesion materials

w. Vibrionic enteritis (*Campylobacter fetus,* subsp. *jejuni, Vibrio cholerae, V. parahaemolyticus).* Recommended precautions. Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities with cultures or potentially infectious clinical materials.

x. *Yersinia pestis*. Biosafety Level 2 practices, containment equipment, and facilities are recommended for all activities involving the handling of potentially infectious clinical materials and cultures. Special care should be taken to avoid the generation of aerosols of infectious materials and during the necropsy of naturally or experimentally infected rodents. Gloves should be worn when handling field-collected or infected laboratory rodents and when there is the likelihood of direct skin contact with infectious agents.

4. Viral Agents

a. Human Herpes Virus. Biosafety Level 2 practices, containment equipment, and facilities are recommended for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that re associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of laboratory-associated infections, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates, or during the necropsy of animals. Primary containment devices constitute the basic barrier protecting personnel from exposure to infectious aerosols.

b. Influenza Virus. Biosafety Level 2 practices and facilities are recommended when receiving and inoculating routine laboratory diagnostic specimens. Autopsy material should be handled in a biological safety cabinet using Biosafety Level 2 procedures.

c. Polio Virus. Biosafety Level 2 practices and facilities are recommended for all activities utilizing known or potentially infectious culture fluids and clinical materials involving known or suspected wild-type strains. All laboratory personnel working directly with the agent must have documented polio vaccination or demonstrated serologic evidence of immunity to three poliovirus types.

9. RELATED DOCUMENTS

- Quest Diagnostics Environmental Health and Safety Manual:
  - *Biohazardous Waste Management Procedure*
  - *Chemical Waste Management and Disposal Procedure*
  - *Post-Exposure Management Policy*
- Quest Diagnostics *Incident Injury and Illness Investigation Report*

10. REFERENCES

- CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 4th edition, September 28, 2000.
- Centers for Disease Control and Prevention. (5th edition; Feb. 2007) *Biosafety in Microbiological and Biomedical Laboratories*. U.S. Government Printing Office, Washington, D.C.

- Centers for Disease Control and Prevention. Sept. 1999. *Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets.* U.S. Government Printing Office, Washington, D.C.
- Isenberg, Henry D. 1998. *Essential Procedures for Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
- Isenberg, Henry D. 1992. *Clinical Microbiology Procedures Handbook*. American Society for Microbiology, Washington, D.C.
- Larone, Davise H. 1995. *Medically Important Fungi.* American Society for Microbiology, Washington, D.C.
- Association for Professionals in Infection Control and Epidemiology, Inc. 1996. *APIC Guideline for Selection and Use of Disinfectants.*
- College of American Pathology. Feb. 21, 2000. Laboratory Accreditation Program Inspection Checklist; Virology Section.
- *Guidance Document For Reporting The Identification Of A Select Agent Or Toxin* (APHIS/CDC), 01/20009

11. REVISION HISTORY

Version	Date	Revision Purpose	Reviser	Approver
		Supersedes SGAH/WAH.M25, GEC.M06 v1		

12. ADDENDA

Appendix A: Laboratory Biosafety Level Criteria
Appendix B: Requirements of PPE and Safety Equipment
Appendix C: Aerosol Transmissible Pathogens – Laboratory

Form revised 3/31/00

ADDENDUM A

LABORATORY BIOSAFETY LEVELS CRITERIA

The four biosafety levels (BSL) for working with infectious agents are described in the CDC-NIH guidelines (*Biosafety in Microbiological and Biomedical Laboratories, U.S. Dept. of Health and Human Services, Fifth Edition, 2007*). Each BSL consists of combinations of equipment, procedures and techniques, and laboratory design that are appropriate for the type of laboratory and infectious agent handled.

NOTE: Each increasing BSL number (BSL 1 to 4) implies increased occupational risk from exposure to an agent and therefore is associated with more stringent control and containment practices.

BSL	Agents	Practices	Primary barriers and Safety Equipment	Facilities (secondary barriers)
1	Not known to consistently cause diseases in healthy adults.	Standard microbiological practices	Not required	Laboratory bench and sink required
2	• Agents associated with human disease • Route 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 coats; gloves; face protection as needed.	BSL-1 plus: • Autoclave available
3	• Indigenous or exotic agents with potential for aerosol transmission • Diseases may have serious or lethal consequences	BSL-2 practice plus: • Controlled access, • Decontamination of all waste, • Decontamination of laboratory clothing before laundering, • Baseline serum test	Primary barriers: • Class I or II BSCs or other physical containment devices used for all open manipulations of agents. PPE: • Protective laboratory clothing; gloves; respiratory protection as needed.	BSL-2 plus: • Physical separation from access corridors • Self-closing, double door access • Exhausted air not recirculated • Negative airflow into laboratory
4	• Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission	BSL-3 practices plus: • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility	Primary barriers: • All procedures conducted in class III BSCs or class I or II BSCs *in combination with* full-body, air-supplied, positive-pressure personnel suit	BSL-3 plus: • Separate building or isolated zone. • Dedicated supply and exhaust, vacuum, and decontamination systems. • Other requirements outlined in text.

NOTE: This is a consolidated list of requirements. For complete information consult the reference listed above.

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ADDENDUM B

REQUIREMENTS OF PPE AND SAFETY EQUIPMENT

Tasks:	Labcoat[a]	Gloves[a]	Face Protection[a]	BSC	Sharps Protection[c]	Fume Hood or Exhaust Systems	Centrifuge with Sealed Safety Caps
Bacterial specimen processing	X	X	X[j]	X[b]	X		X[k]
Blood cultures	X	X		X	X		
Other bacterial cultures	X	R	X[d]				
Bacterial staining	X	R			X[h]		

NOTE: This table indicates general requirements; see the specific sections of this SOP for complete details on PPE and engineering controls. "X" = required; "R" = recommended

a) PPE = Gloves include latex or non-latex substitutes. Face protection includes full-face shield, goggles with surgical mask, or benchtop shield.
b) Biological Safety Cabinet (BSC) is required for the following cultures: bloods, fluids (except urine), stools, tissues, lower respiratory samples, and when handling any culture suspected of containing or growing colonies with morphology typical of any Select Agent, ATP, *Neisseria meningiditis* from a sterile fluid, a *Mycobacterium sp*., or the hyphal form of molds,
c) Indicates that the likelihood of encountering a "contaminated sharp" is high enough to have a sharps container within reach. Includes scalpels, syringes with needles, slides/coverslips.
d) Face shield, goggles with a surgical mask, or benchtop shield is required when there is a risk of splashing, spraying, or spattering. Glass front fume hood is an acceptable alternative for parasitology processing.
e) BSC is required for all fungal specimen processing except "yeast only" cultures; and for mold manipulations (e.g. scotch tape preps or slide culture processing).
f) Virology specimen processing, inoculation, slide preparation or other processes when working with open cultures or specimen containers must be performed under the BSC.
g) Protective clothing such as solid-front or wrap-around gowns are recommended for this task.
h) Slides and coverslips used in microbiological culturing must be disposed into rigid, puncture-proof biohazardous waste containers or sharps containers.
i) Virology microtiter plates must be covered with adhesive sealer prior to centrifuging. Sealed safety caps are also required for spin amplification specimens.
j) Face shield or benchtop shield is required when processing applicable specimen on the benchtop.
k) Use sealed safety caps when centrifuging CSF or other body fluids.

ADDENDUM C

Aerosol Transmissible Pathogens – Laboratory (Cal/OSHA Title 8, Chapter 4, section 5199)

This addendum contains a list of agents that, when reasonably anticipated to be present, require a risk assessment and establishing a biosafety plan that includes appropriate control measures as identified in the standard.

Adenovirus (in clinical specimens and in cultures or other materials derived from clinical specimens)

Arboviruses, unless identified individually elsewhere in this list (large quantities or high concentrations* of arboviruses for which CDC recommends BSL-2, e.g., dengue virus; potentially infectious clinical materials, infected tissue cultures, animals, or arthropods involving arboviruses for which CDC recommends BSL-3 or higher, e.g., Japanese encephalitis, West Nile virus, Yellow Fever)

Arenaviruses (large quantities or high concentrations of arenaviruses for which CDC recommends BSL-2, e.g., Pichinde virus; potentially infectious clinical materials, infected tissue cultures, animals, or arthropods involving arenaviruses for which CDC recommends BSL-3 or higher, e.g., Flexal virus)

Bacillus anthracis (activities with high potential for aerosol production**, large quantities or high concentrations, screening environmental samples from *B. anthracis* - contaminated locations)

Blastomyces dermatitidis (sporulating mold-form cultures, processing environmental materials known or likely to contain infectious conidia)

Bordetella pertussis (aerosol generation, or large quantities or high concentrations)

Brucella abortus, B. canis, B. "maris", B. melitensis, B. suis (cultures, experimental animal studies, products of conception containing or believed to contain pathogenic *Brucella* spp.)

Burkholderia mallei, B. pseudomallei (potential for aerosol or droplet exposure, handling infected animals, large quantities or high concentrations)

Cercopithecine herpesvirus (see Herpesvirus simiae)

Chlamydia pneumoniae (activities with high potential for droplet or aerosol production, large quantities or high concentrations)

Chlamydia psittaci (activities with high potential for droplet or aerosol production, large quantities or high concentrations, non-avian strains, infected caged birds, necropsy of infected birds and diagnostic examination of tissues or cultures known to contain or be potentially infected with *C. psittaci* strains of avian origin)

Chlamydia trachomatis (activities with high potential for droplet or aerosol production, large quantities or high concentrations, cultures of lymphogranuloma venereum (LGV) serovars, specimens known or likely to contain *C. trachomatis*)

Clostridium botulinum (activities with high potential for aerosol or droplet production, large quantities or high concentrations)

Coccidioides immitis, C. posadasii (sporulating cultures, processing environmental materials known or likely to contain infectious arthroconidia, experimental animal studies involving exposure by the intranasal or pulmonary route)

Corynebacterium diphtheriae

Coxiella burnetti (inoculation, incubation, and harvesting of embryonated eggs or cell cultures; experimental animal studies, animal studies with infected arthropods, necropsy of infected animals, handling infected tissues)
Crimean-Congo haemorrhagic fever virus
Cytomegalovirus, human (viral production, purification, or concentration)
Eastern equine encephalomyelitis virus (EEEV) (clinical materials, infectious cultures, infected animals or arthropods)
Ebola virus
Epstein-Barr virus (viral production, purification, or concentration)
Escherichia coli, shiga toxin-producing only (aerosol generation or high splash potential)
Flexal virus
Francisella tularensis (suspect cultures—including preparatory work for automated identification systems, experimental animal studies, necropsy of infected animals, high concentrations of reduced-virulence strains)
Guanarito virus
Haemophilus influenzae, type b
Hantaviruses (serum or tissue from potentially infected rodents, potentially infected tissues, large quantities or high concentrations, cell cultures, experimental rodent studies)
Helicobacter pylori (homogenizing or vortexing gastric specimens)
Hemorrhagic fever -- specimens from cases thought to be due to dengue or yellow fever viruses or which originate from areas in which communicable hemorrhagic fever are reasonably anticipated to be present
Hendra virus
Hepatitis B, C, and D viruses (activities with high potential for droplet or aerosol generation, large quantities or high concentrations of infectious materials)
Herpes simplex virus 1 and 2
Herpesvirus simiae (B-virus) (consider for any material suspected to contain virus, mandatory for any material known to contain virus, propagation for diagnosis, cultures)
Histoplasma capsulatum (sporulating mold-form cultures, propagating environmental materials known or likely to contain infectious conidia)
Human herpesviruses 6A, 6B, 7, and 8 (viral production, purification, or concentration)
Influenza virus, non-contemporary human (H2N2) strains, 1918 influenza strain, highly pathogenic avian influenza (HPAI) (large animals infected with 1918 strain and animals infected with HPAI strains in ABSL-3 facilities, loose-housed animals infected with HPAI strains in BSL-3-Ag facilities)
Influenza virus, H5N1 - human, avian
Junin virus
Kyasanur forest disease virus
Lassa fever virus
Legionella pneumophila, other legionella-like agents (aerosol generation, large quantities or high concentrations)
Lymphocytic choriomeningitis virus (LCMV) (field isolates and clinical materials from human cases, activities with high potential for aerosol generation, large quantities or high concentrations, strains lethal to nonhuman primates, infected transplantable tumors, infected hamsters)
Machupo virus
Marburg virus
Measles virus
Monkeypox virus (experimentally or naturally infected animals)

Mumps virus
Mycobacterium tuberculosis complex (M. africanum, M. bovis, M. caprae, M. microti, M. pinnipedii, M. tuberculosis (aerosol-generating activities with clinical specimens, cultures, experimental animal studies with infected nonhuman primates)
Mycobacteria spp. other than those in the *M. tuberculosis* complex and *M. leprae* (aerosol generation)
Mycoplasma pneumoniae
Neisseria gonorrhoeae (large quantities or high concentrations, consider for aerosol or droplet generation)
Neisseria meningitidis (activities with high potential for droplet or aerosol production, large quantities or high concentrations)
Nipah virus
Omsk hemorrhagic fever virus
Parvovirus B19
Prions (bovine spongiform encephalopathy prions, only when supported by a risk assessment)
Rabies virus, and related lyssaviruses (activities with high potential for droplet or aerosol production, large quantities or high concentrations)
Retroviruses, including Human and Simian Immunodeficiency viruses (HIV and SIV) (activities with high potential for aerosol or droplet production, large quantities or high concentrations)
Rickettsia prowazekii, Orientia (Rickettsia) tsutsuagmushi, R. typhi (R. mooseri), Spotted Fever Group agents (*R. akari, R. australis, R. conorii, R. japonicum, R. rickettsii,* and *R. siberica*) (known or potentially infectious materials; inoculation, incubation, and harvesting of embryonated eggs or cell cultures; experimental animal studies with infected arthropods)
Rift valley fever virus (RVFV)
Rubella virus
Sabia virus
Salmonella spp. other than *S. typhi* (aerosol generation or high splash potential)
Salmonella typhi (activities with significant potential for aerosol generation, large quantities)
SARS coronavirus (untreated specimens, cell cultures, experimental animal studies)
Shigella spp. (aerosol generation or high splash potential)
Streptococcus spp., group A
Tick-borne encephalitis viruses (Central European tick-borne encephalitis, Far Eastern tick-borne encephalitis, Russian spring and summer encephalitis)
Vaccinia virus
Varicella zoster virus
Variola major virus (Smallpox virus)
Variola minor virus (Alastrim)
Venezuelan equine encephalitis virus (VEEV) (clinical materials, infectious cultures, infected animals or arthropods)
West Nile virus (WNV) (dissection of field-collected dead birds, cultures, experimental animal and vector studies)
Western equine encephalitis virus (WEEV) (clinical materials, infectious cultures, infected animals or arthropods)
Yersinia pestis (antibiotic resistant strains, activities with high potential for droplet or aerosol production, large quantities or high concentrations, infected arthropods, potentially infected animals)

* 'Large quantities or high concentrations' refers to volumes or concentrations considerably in excess of those typically used for identification and typing activities. A risk assessment must be performed to determine if the quantity or concentration to be used carries an increased risk, and would therefore require aerosol control.

** 'activities with high potential for aerosol generation' include centrifugation