HOW TO SAFELY WORK WITH INFECTIOUS VETERINARY DIAGNOSTIC SAMPLES

Centers for Disease Control and Prevention, National Center for Emerging and Infectious Diseases, Division of Scientific Resources

DISCLAIMER

The findings and conclusions in this presentation are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention and Federal Select Agent Program.

AGENDA

- Risk groups
- Zoonotic pathogens
- Biosafety program
- Containment
- Risk assessment
- Facility practices and procedures
- Working with diagnostic samples
- Disinfection

RISK GROUP CLASSIFICATIONS

BASED ON NIH AND WHO GUIDELINES

RG 1

Not associated with disease in healthy adult humans

No or low individual and community risk

RG 2

Associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available

Moderate individual risk, low community risk

RG 3

Associated with serious or lethal human disease for which preventive or therapeutic interventions may be available

High individual risk, low community risk

RG 4

Likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available

High individual and community risk

HHS AND USDA SELECT AGENTS AND TOXINS 7CFR PART 331, 9 CFR PART 121, AND 42 CFR PART 73

https://www.selectagents.gov/sat/list.htm

HHS Select Agents and Toxins

1. Abrin [6]
2. *Bacillus cereus* Biovar *anthracis* [1]
3. Botulinum neurotoxins [1][6]
4. Botulinum neurotoxin producing species of *Clostridium* [1]
5. Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$) [6]
6. *Coxiella burnetii*
7. Crimean-Congo haemorrhagic fever virus
8. Diacetoxyscirpenol [6]
9. Eastern Equine Encephalitis virus [4][5]
10. Ebola virus [1]
11. *Francisella tularensis* [1]
12. Lassa fever virus
13. Lujo virus
14. Marburg virus [1]
15. Monkeypox virus [4]
16. Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
17. Ricin [6]
18. *Rickettsia prowazekii*
19. SARS-associated coronavirus (SARS-CoV) [5]
20. Saxitoxin [6]

South American Haemorrhagic Fever viruses:

21. Chapare
22. Guanarito
23. Junin
24. Machupo
25. Sabia

26. Staphylococcal enterotoxins (subtypes A,B,C,D,E) [6]
27. T-2 toxin [6]
28. Tetrodotoxin [6]

Tick-borne encephalitis complex (flavi) viruses:

29. Far Eastern subtype [5]
30. Siberian subtype [5]

31. Kyasanur Forest disease virus [5]
32. Omsk hemorrhagic fever virus [5]
33. Variola major virus (Smallpox virus) [1]
34. Variola minor virus (Alastrim) [1]
35. *Yersinia pestis* [1]

Overlap Select Agents and Toxins

36. *Bacillus anthracis* [1]
37. *Bacillus anthracis* Pasteur strain
38. *Brucella abortus*
39. *Brucella melitensis*
40. *Brucella suis*
41. *Burkholderia mallei* [1]
42. *Burkholderia pseudomallei* [1]
43. Hendra virus
44. Nipah virus
45. Rift Valley fever virus
46. Venezuelan equine encephalitis virus [4][5]

USDA Select Agents and Toxins

47. African horse sickness virus
48. African swine fever virus
49. Avian influenza virus [4]
50. Classical swine fever virus [5]
51. Foot-and-mouth disease virus [1][5]
52. Goat pox virus
53. Lumpy skin disease virus
54. *Mycoplasma capricolum* [4]
55. *Mycoplasma mycoides* [4]
56. Newcastle disease virus [3][4]
57. Peste des petits ruminants virus
58. Rinderpest virus [1]
59. Sheep pox virus
60. Swine vesicular disease virus [5]

USDA Plant Protection And Quarantine (PPQ) Select Agents and Toxins

61. *Coniothyrium glycines* (formerly *Phoma glycinicola* and *Pyrenochaeta glycines*)
62. *Peronosclerospora philippinensis* *(Peronosclerospora sacchari)*
63. *Ralstonia solanacearum* [7]
64. *Rathayibacter toxicus*
65. *Sclerophthora rayssiae* [7]
66. *Synchytrium endobioticum*
67. *Xanthomonas oryzae*

NON-SELECT AGENT ZOONOTIC PATHOGENS

Sheep and goats:
- Orf (contagious ecthyma)

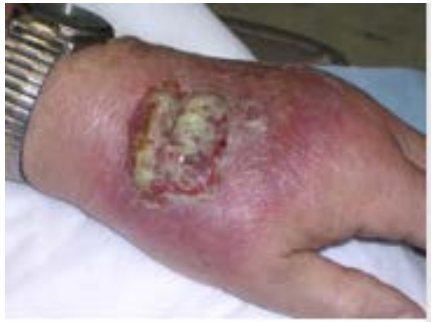
Cows:
- *Mycobacterium bovis*

NHPs:
- Herpesvirus B
- Hepatitis B
- *Mycobacterium tuberculosis*

Birds:
- *Chlamydia psittaci*

Rodents:
- LCMV (lymphocytic choriomeningitis virus)

All species:
- Rabies



Orf virus infection on the hand of a person with a weak immune system.

https://www.cdc.gov/poxvirus/orf-virus/people.html

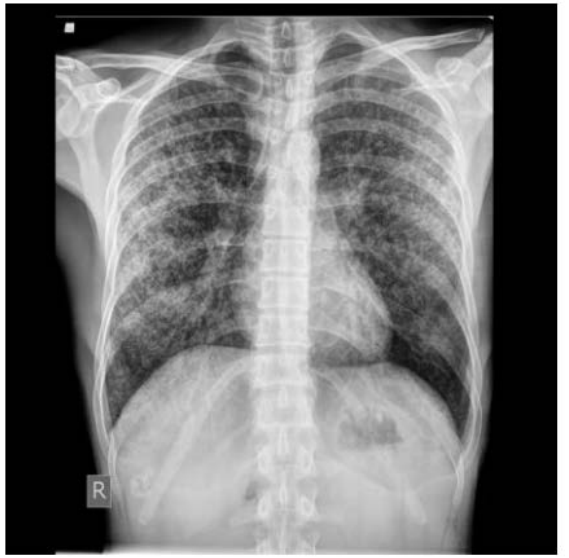


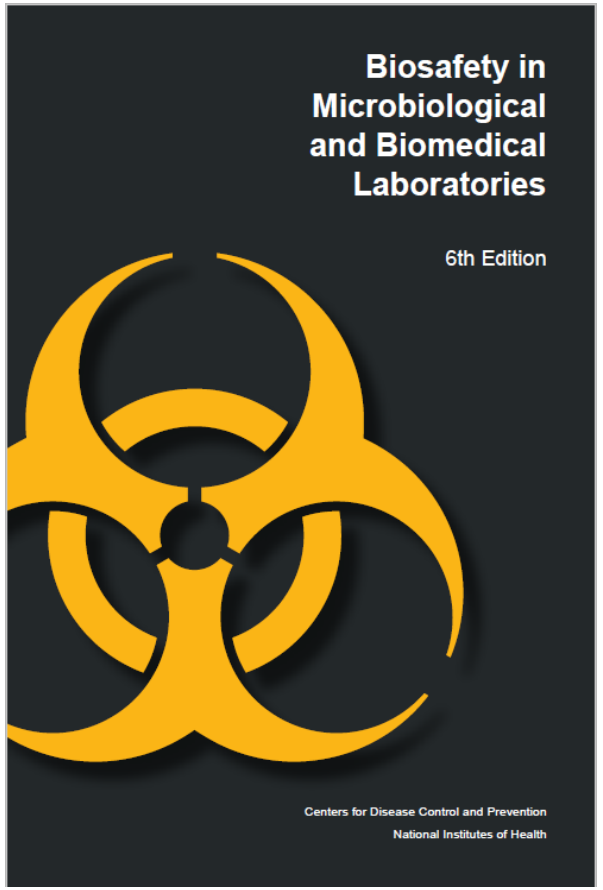
Image | Radiopaedia.org

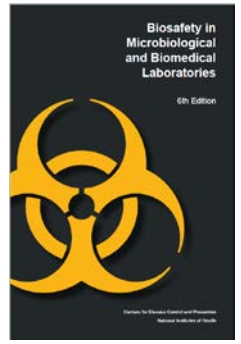
And so many more……

BIOSAFETY PROGRAM

A fundamental objective of any biosafety program is the containment of potentially hazardous biological agents and toxins.

- The term “containment” is used to describe safe methods for managing infectious materials in the laboratory environment where they are being handled or maintained.
- **The purpose of containment is to reduce or eliminate exposure** of laboratory workers, other persons, and the outside environment to potentially hazardous agents.

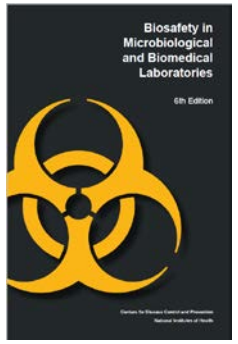




PRIMARY CONTAINMENT

Primary containment, the protection of personnel and the immediate laboratory environment from exposure to infectious agents, is provided by both good microbiological technique and the use of appropriate safety equipment.

- **The BSC is the standard device used to provide containment of hazardous biological agents and toxins when conducting microbiological activities.**
- Additional primary containment devices may include:
  - Sealed rotors and centrifuge safety cups which prevent aerosols, droplets, and leakage of hazardous biological agents and toxins that may result during centrifugation.
  - Sealed containers provide containment for transfers between laboratories.



PERSONAL PROTECTIVE EQUIPMENT

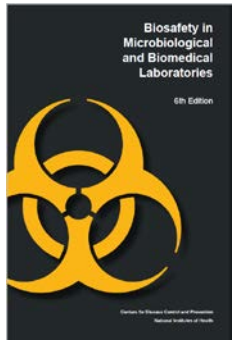
Personal protective equipment (PPE) helps protect the user's body from injury from a variety of sources (e.g., physical, electrical, heat, noise, chemical) or potential exposure to biological hazards and airborne particulate matter.

- Examples: gloves, coats, gowns, shoe covers, closed-toe laboratory footwear, respirators, face shields, safety glasses, goggles, or ear plugs.

PPE is usually used in combination with other biosafety controls (e.g., BSCs, centrifuge safety cups, and small animal caging systems) that contain the hazardous biological agents and toxins, animals, or materials being handled.

In situations where a BSC cannot be used, **PPE may become the primary barrier between personnel and the hazardous biological agents and toxins**.

- Examples include fieldwork, resource-limited settings, certain animal studies, animal necropsy, and activities relating to operations, maintenance, service, or support of the laboratory facility.



SECONDARY CONTAINMENT

Secondary containment, the protection of the environment external to the laboratory from exposure to infectious materials, is provided by a combination of facility design and operational practices. Therefore, the three elements of containment include laboratory practice and technique, safety equipment, and facility design.

Such design features may include, but are not limited to the following:

- Ventilation strategies to ensure containment of the hazards;
- Effluent decontamination systems; and
- Specialized building/suite/laboratory configurations, including: controlled access zones to support the separation of the laboratory from office and public spaces;
- Anterooms; and
- Airlocks.

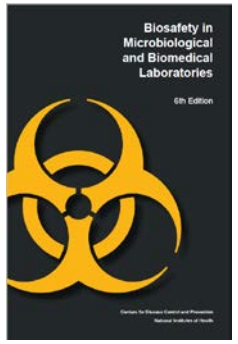
Table 1. Summary of Laboratory Biosafety Levels (BSLs)

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
1	Well-characterized agents not known to consistently cause disease in immunocompetent adult humans and present minimal potential hazard to laboratory personnel and the environment.	Standard microbiological practices	No primary barriers required; protective laboratory clothing; protective face, eyewear, as needed	Laboratory doors; sink for handwashing; laboratory bench; windows fitted with screens; lighting adequate for all activities
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory
4	Dangerous and exotic agents that pose high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that are frequently fatal, for which there are no vaccines or treatments; and related agents with unknown risk of transmission	Clothing change before entry; daily inspections of essential containment and life support systems; all wastes decontaminated prior to removal from laboratory; shower on exit	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls;[b] gloves;[b] full-body, air-supplied, positive-pressure suit[c]	Entry sequence; entry through airlock with airtight doors;[c] walls, floors, ceilings form sealed internal shell; dedicated, non-recirculating ventilation system required; double-door, pass-through autoclave required

a. Each successive BSL contains the recommendations of the preceding level(s) and the criteria in the cell.
b. Applies to Cabinet Laboratory
c. Applies to Suit Laboratory

BMBL 6th Ed. pg 68-69

CLINICAL LABORATORIES



Clinical laboratories routinely work with unknown specimens and specimens that have the potential to be infected with multiple pathogens; as such, the occupational risks in a clinical laboratory environment differ from those of a research or teaching laboratory. Most public and animal health clinical laboratories use BSL-2 facility, engineering, and biosafety practices.

Clinical diagnostic laboratory personnel may not know what infectious agent or other hazard(s) exist in the specimen they handle and process.

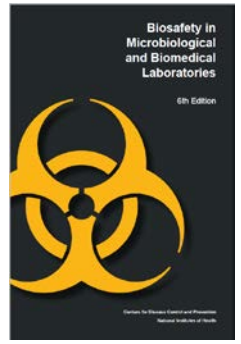
Final determination on the combination of containment measures required to address the relevant biosafety risk present at a facility should be based on a **comprehensive biosafety risk assessment**.

TABLE 27.1 A Matrix Commonly Used in the Risk Assessment Process When Determining the Level of Risk

Probability of the event occurring					
Severity of the outcome	**Frequent**	**Likely**	**Occasional**	**Seldom**	**Unlikely**
Catastrophic	Extremely high	Extremely high	High	High	Moderate
Critical	Extremely high	High	High	Moderate	Low
Marginal	High	Moderate	Moderate	Low	Low
Negligible	Moderate	Low	Low	Low	Low

Fox, James G. *Laboratory animal medicine*. Elsevier, 2015. pg 1299

CONDUCTING RISK ASSESSMENTS IN A CLINICAL LABORATORY ENVIRONMENT



The assessment team should determine what hazards may exist and the risks associated with those hazards.

- When the agent hazards are unknown, it may be helpful for clinical laboratories to monitor current disease outbreaks and compile lists of commonly encountered pathogens for a population, region, or specimen type.

To help structure biological risk assessments, clinical laboratories should consider what procedures or activities will be performed, where the work will be performed, who will perform the work, and what undesirable events could occur.

It is also essential to evaluate the potential routes of transmission of the suspected infectious agent (i.e., inhalation of aerosols, ingestion, percutaneous inoculation from sharps or non-intact skin, and direct mucous membrane contact from splashes or droplets).

THE STANDARD RISK ASSESSMENT FORMULA

Severity x Likelihood = Risk

Criteria for Probability Classifications (per OADLSS SOP)	
FREQUENT	> 50% OF TESTING
PROBABLE	11 - 50% OF TESTING
OCCASIONAL	1 - 10% OF TESTING
REMOTE	< 1% OF TESTING
IMPROBABLE	STATISTICALLY INSIGNIFICANT

Criteria for Severity Classification (per OLSS SOP)	
Catastrophic	Potential for appreciable material cost
	National or international impact
	Work with risk group 4 pathogens
Critical	Potential for appreciable material cost
	Scope of impact reaching outside CDC
	Work with risk group 3 pathogens
Serious	Potential for appreciable material cost
	Scope of impact reaching outside the branch
	Work with risk group 2 pathogens
Minor	Potential for minor material cost
	Scope of impact limited to the team or branch
	Work with risk group 1 organisms
Negligible	Potential for minor material cost
	Scope of impact limited to the team or branch
	Work with nucleic acid extracts or other inactivated material

LIKELIHOOD/PROBABILITY

Criteria for Probability Classifications (per OLSS SOP)	
FREQUENT	> 50% OF TESTING
PROBABLE	11 - 50% OF TESTING
OCCASIONAL	1 - 10% OF TESTING
REMOTE	< 1% OF TESTING
IMPROBABLE	STATISTICALLY INSIGNIFICANT

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REMOTE	< 1% OF TESTING
IMPROBABLE	STATISTICALLY INSIGNIFICANT

CONSEQUENCE SEVERITY

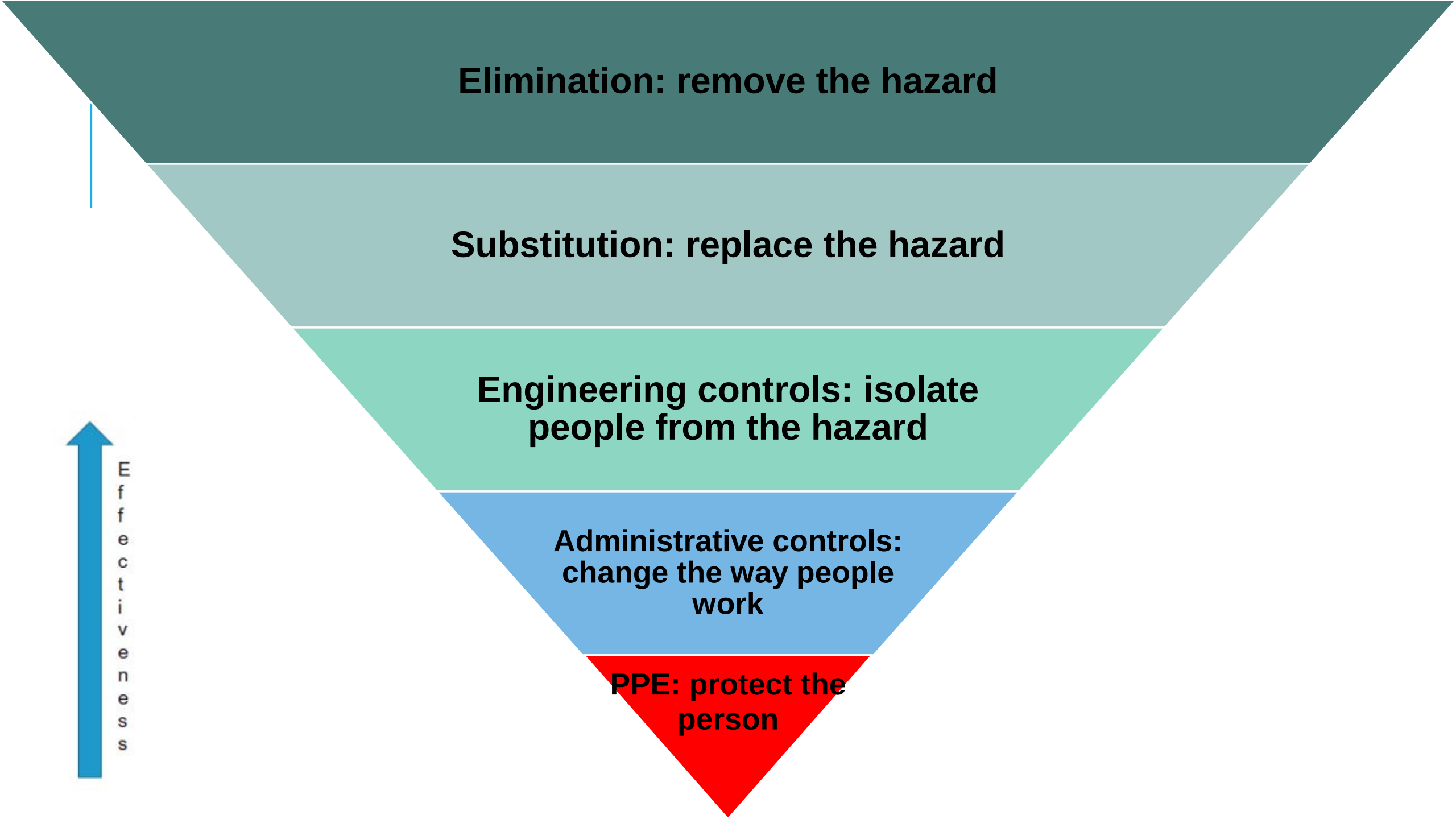
Criteria for Severity Classification (per OLSS SOP)	
Catastrophic	Potential for appreciable material cost
	National or international impact
	Work with risk group 4 pathogens
Critical	Potential for appreciable material cost
	Scope of impact reaching outside CDC
	Work with risk group 3 pathogens
Serious	Potential for appreciable material cost
	Scope of impact reaching outside the branch
	Work with risk group 2 pathogens
Minor	Potential for minor material cost
	Scope of impact limited to the team or branch
	Work with risk group 1 organisms
Negligible	Potential for minor material cost
	Scope of impact limited to the team or branch
	Work with nucleic acid extracts or other inactivated material

WHY DO A RISK ASSESSMENT?

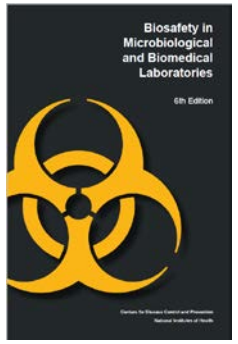
Occupational risk assessment: systematic process of evaluating risks from identified occupational hazards associated with a procedure/process in order to mitigate such risks as well as determining if a risk is acceptable or not.

When to do a risk assessment:

- New procedure
- In response to an incident
- Annually


Elimination: remove the hazard
Substitution: replace the hazard
Engineering controls: isolate people from the hazard
Administrative controls: change the way people work
PPE: protect the person
Effectiveness

FACILITY PRACTICES AND PROCEDURES



Established facility-specific best practices and procedures are essential to support the implementation and sustainability of a successful biosafety program.

Persons working in facilities that handle and store hazardous biological agents and toxins must be able to properly identify all potential hazards and be trained and proficient in necessary safe practices and procedures.

Strict adherence to documented laboratory best practices and procedures is an essential element of a robust biosafety program.

All facilities should develop and implement a biosafety program that identifies the hazards and specifies risk mitigation strategies to eliminate or reduce the likelihood of exposures and unintentional releases of hazardous materials.

HANDLING OF TISSUES WITHIN PRIMARY CONTAINMENT

Biosafety cabinet – Class II BSCs are the primary containment devices that protect the worker, product, and environment from exposure to microbiological agents.

Down draft table – Ventilated workstation that pulls air down and away from personnel.

Centrifuge cups

Sealed containers

PPE is still important!!!!

BEST PRACTICES WHEN UNABLE TO HANDLE WITHIN PRIMARY CONTAINMENT

According the BMBL, when a procedure cannot be performed within a primary barrier, a combination of personal protective equipment and other containment devices must be used.

PPE choice depends on risk/possible exposure:

- Gloves (1 vs 2 layers) – Protects skin
- Scrubs/Gown/Disposable coveralls – Protects skin and clothing
- Face shield - Protects against splashes
- Safety glasses/Safety goggles – Protects against splashes
- Respirator vs PAPR – Protects against aerosols
- Dedicated shoes/Shoe covers

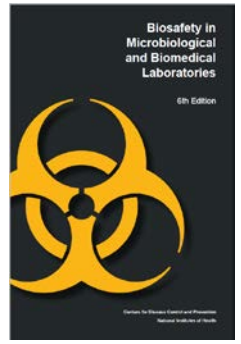
Other containment devices:

- Isolated zone
- Sealed containers

APPROPRIATE PPE WHEN PERFORMING NECROPSIES, SURGICAL PROCEDURES, AND DIAGNOSTIC TESTING

Depends on risk assessment:

- Possible exposure to infectious agents?
  - What agent?
    - Select agent
    - Zoonotic agent
  - Severity of disease?
    - No disease
    - Low risk of disease – Therapeutic available
    - Severe disease – Therapeutic available
    - Severe disease – No treatment
  - Route of transmission?
    - Percutaneous
    - Mucous membrane
    - Inhalation
    - Ingestion
    - Environmental



WHAT PPE/CONTAINMENT PRACTICES SHOULD YOU USE?

You receive a placenta from a sheep that recently aborted….

- Possible exposure to infectious agents?
  - What agent?
    - *Coxiella burnetii* (Q fever)
  - Severity of disease?
    - Range from subclinical to severe – Therapeutics available
  - Route of transmission?
    - Inhalation***
    - Others…….

https://www.cdc.gov/vhf/rvf/exposure/index.html

According to BMBL pg 239:

Q fever is the second most commonly reported Laboratory-associated infection (LAI) in Pike's compilation with outbreaks involving 15 or more persons recorded in several institutions. Infectious aerosols are the most likely route of LAI.

BSL-3 practices and facilities are recommended for activities involving the inoculation, incubation, and harvesting of *C. burnetii*, the necropsy of infected animals, and the manipulation of infected tissues. BSL-2 practices and facilities are recommended for nonpropagative laboratory procedures, including serological examinations and staining of impression smears.

Table 1. Summary of Laboratory Biosafety Levels (BSLs)

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory

BMBL 6th Ed. pg 68-69

DISINFECTION

Always clean and sanitize your workspace and any instruments used with an APPROPRIATE **disinfectant** for the APPROPRIATE **contact time**.

TABLE. Chemical compounds used for disinfection, effectiveness of chemical disinfectants and selected products against certain organisms, and selected properties of chemical disinfectants that should be considered when used for cleaning and disinfection

Chemical compounds	Chlorine* 0.01%–5%	Iodine iodophor 0.5%–5%	Chlorhexidine 0.05%–0.5%	Alcohol† 70%	Oxidizing agents 0.2%–3%	Phenol 0.2%–3%	Quaternary ammonium 0.1%–2%
Selected products	Clorox®	Tincture/ Provodine	Nolvasan®	Rubbing alcohol	Virkon-S®	pHisoHex®	Roccal-D®
Effectiveness of chemical disinfectants against certain organisms§							
Bactericidal	Good	Good	Good	Good	Good	Good	Good
Bacterial spores	Good¶	Poor	Poor	Poor¶	Fair to good	Poor	Poor
Virucidal	Good	Good	Poor	Fair	Good	Poor**	Poor
Envelope viruses	Yes	Yes	Limited	Yes	Yes	Limited	Limited
Nonenvelope viruses	Yes	Limited	No	No	Yes	No	No
Fungicidal	Good	Fair	Fair to good	Good	Fair	Fair	Fair
Protozoal parasites	Fair (concentrated)	Poor	Poor	Poor	Poor	Poor	Fair (ammonia)
Properties of chemical disinfectants††							
Effectiveness in organic matter	Poor	Poor	Fair	Poor	Poor	Good	Poor
Inactivated by soap	No	Yes	No	No	No	No	Yes
Effective in hard water	Yes	No	Yes	Yes	Yes	Yes	No
Residual activity	Poor	Poor	Good	Fair	Poor	Poor	Fair

Source: Adapted from the Nebraska Cooperative Extension and the U.S. Department of Agriculture, 2003.

* Bleach should be mixed fresh daily and replaced whenever contaminated with organic matter (1:32 dilution of 5.75% solution provides >1,500 ppm chlorine).

† Rubbing alcohol is flammable.

§ Effectiveness as a bactericidal, virucidal, or fungicidal agent and effectiveness in eliminating bacterial spores and protozoal parasites: good = effective; fair = moderate effect; and poor = inferior effect. Effectiveness in eliminating envelope and nonenvelope viruses: yes = effective; limited = moderate effect; and no = not effective.

¶ Alcohol synergistically potentiates the sporicidal effect of hypochlorites (chlorine). Mix 5.75% solution of hypochlorite 1:1 with 50% ethyl alcohol/water. Mix fresh at the time of use and provide contact time of ≥30 minutes.

** The effectiveness of 2-phenylphenol (ortho-phenylphenol) is fair.

†† Effectiveness in organic matter: good = effective; fair = moderate effect; and poor = inferior effect. Inactivated by soap and effective in hard water: yes = chemical compound has this property; no = chemical compound does not have this property. Residual activity: good = chemical compound has residual activity; fair = moderate residual activity; and poor = inferior residual activity.

https://www.cdc.gov/mmwr/preview/mmwrhtml/rr5404a2.htm

IN CONCLUSION

Determine exposure risk

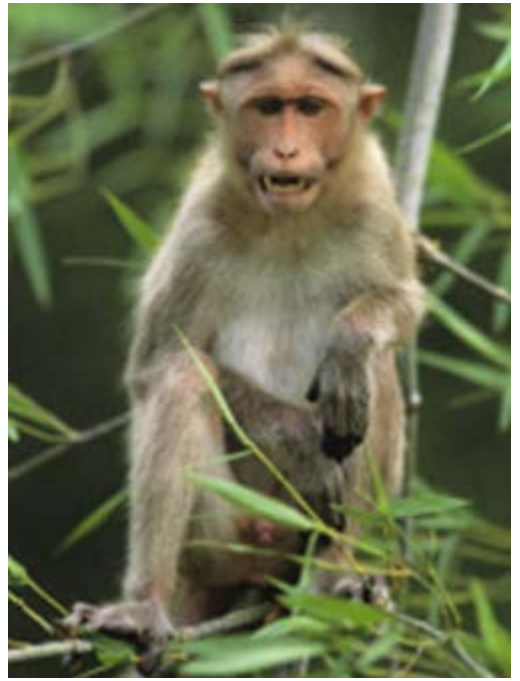
Establish SOPs

Use appropriate containment devices and PPE

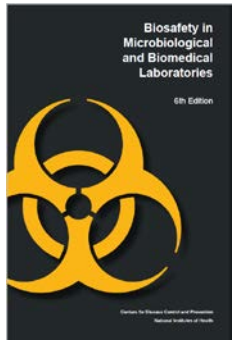
Report any possible exposures to your immediate supervisor and Responsible Official.

You receive a blood sample from a macaque….

1. Possible exposure to infectious agents?
   A. Yes
   B. No
2. What agent should you be **most** concerned about?
   A. *Mycobacterium tuberculosis*
   B. *Chlamydia psittaci*
   C. *Francisella tularensis*
   D. Herpesvirus B
3. What is the primary route of transmission?
   A. Percutaneous
   B. Inhalation
   C. Mucous membrane
   D. A and C
4. What biosafety level should the blood sample be handled at?
   A. ABSL 1
   B. ABSL 2
   C. ABSL 3
   D. ABSL 4



https://www.cdc.gov/herpesbvirus/index.html



ACCORDING TO THE BMBL 6TH ED.

Exposure via mucous membranes or skin breaks provides this agent access to a new host, whether the virus is being shed from a macaque or human, or is present in or on contaminated cells, tissues, or surfaces. B virus is **not** generally found in serum or blood, but these products obtained through venipuncture should be handled carefully because contamination of needles via skin can occur.

BSL-3 practices are recommended for handling diagnostic materials with possible B virus. **BSL-2 practices and facilities are suitable for all activities involving the use or manipulation of tissues, cells, blood, or serum from macaques with appropriate personal protective equipment.** pg 255

All macaques regardless of their origin should be considered potentially infected. Animals with no detectable antibody are not necessarily B virus-free. Macaques should be handled with strict barrier precaution protocols and injuries should be tended immediately according to the recommendations of the B Virus Working Group led by NIH and CDC.

Table 1. Summary of Laboratory Biosafety Levels (BSLs)

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
2	Agents associated with human disease and pose moderate hazards to personnel and the environment	Limited access; occupational medical services including medical evaluation, surveillance, and treatment, as appropriate; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination process needed for laboratory equipment	BSCs or other primary containment device used for manipulations of agents that may cause splashes or aerosols; protective laboratory clothing; other PPE, including respiratory protection, as needed	Self-closing doors; sink located near exit; windows sealed or fitted with screens; autoclave available

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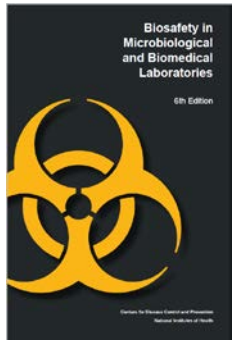
Additional information:
https://www.cdc.gov/herpesbvirus/index.html

A rabbit was found dead and submitted for necropsy….

https://www.cdc.gov/tularemia/index.html

1. Possible exposure to infectious agents?
   A. Yes
   B. No
2. What agent should you be **most** concerned about?
   A. *Mycobacterium tuberculosis*
   B. *Chlamydia psittaci*
   C. *Francisella tularensis*
   D. Herpesvirus B
3. What is the primary route of transmission?
   A. Ingestion
   B. Inhalation
   C. Percutaneous
   D. All the above
4. What biosafety level should the necropsy be conducted at?
   A. ABSL 1
   B. ABSL 2
   C. ABSL 3
   D. ABSL 4



ACCORDING TO THE BMBL 6TH ED.

The agent may be present in lesion exudates, respiratory secretions, CSF, blood or lymph node aspirates from patients, tissues from infected animals, fluids from infected animals, and fluids from infected arthropods. Direct contact of skin or mucous membranes with infectious materials, accidental parenteral inoculation, ingestion, and exposure to aerosols and infectious droplets have resulted in infection.

BSL-3 and ABSL-3 practices, containment equipment, and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies. pg 166

BSL	Agents	Special Practices[a]	Primary Barrier and Personal Protective Equipment[a]	Facilities (Secondary Barriers)[a]
3	Indigenous or exotic agents; may cause serious or potentially lethal disease through the inhalation route of exposure	Access limited to those with need to enter; viable material removed from laboratory in primary and secondary containers; opened only in BSL-3 or ABSL-3 laboratories; all procedures with infectious materials performed in a BSC	BSCs for all procedures with viable agents; solid front gowns, scrubs, or coveralls; two pairs of gloves, when appropriate; protective eyewear, respiratory protection, as needed	Physical separation from access corridors; access through two consecutive self-closing doors; hands-free sink near exit; windows are sealed; ducted air ventilation system with negative airflow into laboratory; autoclave available, preferably in laboratory

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Additional information:

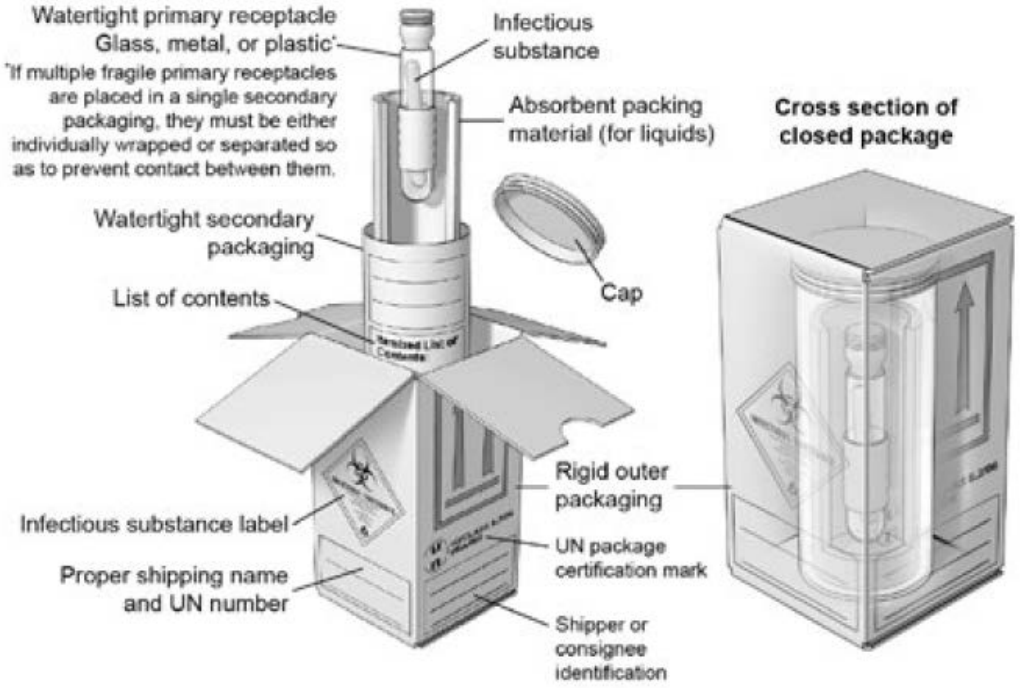
https://www.cdc.gov/tularemia/index.html

TRANSPORTATION OF INFECTIOUS MATERIAL

Category A Infectious Substance
UN 2814 (infectious toward humans or both animals and humans)
UN 2900 (infectious towards Animals only)

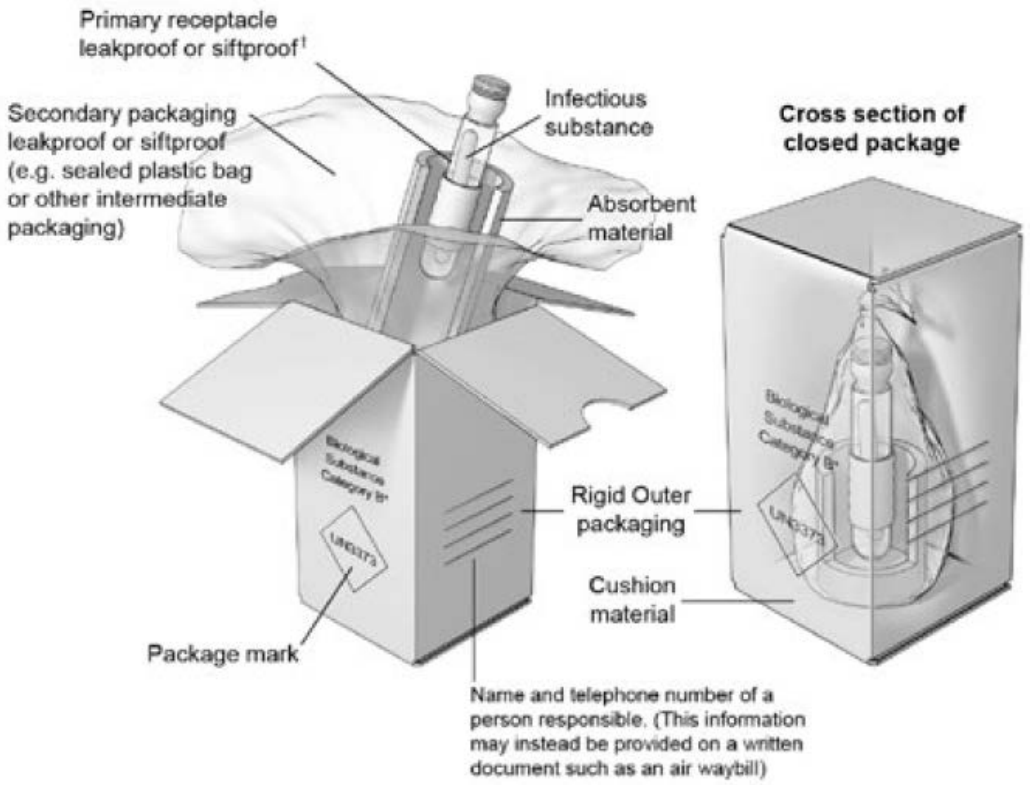
- Capable of causing permanent disability or life-threatening or fatal disease to otherwise healthy humans or animals.
- Triple packaging system:
  1. Watertight primary receptacle
     - Absorbent material
  2. Watertight secondary packaging
  3. Rigid outer packaging of adequate strength for its capacity, mass, and intended use
- Each surface of the external dimension of the packaging must be 100 mm (3.9in) or more.
- Drop test 1.2m (3.9ft), water-spray test, pressure change of 95 kPa (0.95bar, 14psi), temperatures in the range of -40°C to +55°C.

Figure 1. A Category A UN Standard Triple Packaging



TRANSPORTATION OF INFECTIOUS MATERIAL

Figure 2. A Category B Non-specification Triple Packaging



Biological specimen, Category B
UN 3373 (non-infectious)

- A Category B infectious substance does not cause permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs.
- Triple packaging system:
  1. Leak proof primary receptacle
     - Absorbent material
  2. Leak proof secondary packaging
  3. Rigid outer packaging
- At least one surface of the outer packaging must have a minimum dimension of 100mm by 100mm (3.9in).
- Internal pressure differential of 95 kPa.
- Capable of passing a 1.2m (3.9ft) drop test.