SAND2012-4396C

Controlling Laboratory Biorisks

JORDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

International Biological Threat Reduction Program

June 2012

SAND **2012-4396C** Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Contents

Instructor Bios

Eric Cook, MPH, CBSP

Eric Cook is a Senior Member of the Technical Staff in the International Biological Threat Reduction Program at Sandia National Laboratories where his work focuses on promoting global laboratory biorisk management. He serves as one of the program's lead trainers. Eric served as the Biosafety Officer for Dartmouth College in Hanover, NH for five years where he managed the Biosafety Program and Institutional Biosafety Committee. He also served as a member of the Animal Care and Use committee. Prior to working at Dartmouth, Eric was an Assistant Biosafety Officer at the Massachusetts Institute of Technology for six years. While at MIT, Eric completed his Master's Degree in Public Health at Boston University with a focus on Environmental Health. Eric has a B.S.in Molecular Biology from Brigham Young University where he worked in a recombinant DNA lab for two years assisting with work in phylogenetics. He is certified by the National Registry of Certified Microbiologists as a Specialist Microbiologist in Biological Safety Microbiology and by the American Biological Safety Association as a Certified Biological Safety Professional. Eric has particular expertise in dangerous goods and infectious substance shipping. He worked for three years at Saf-T-Pak, Inc. developing training programs and packaging for shipping infectious substances. He also speaks Korean and worked as a training coordinator and translator at the LG Electronics training center in Songtan, South Korea. encook@sandia.gov

Cecelia V. Williams, PhD

Cecelia V. Williams is a Principal Member of the Technical Staff in the International Biological Threat Reduction Program at Sandia National Laboratories. Over her 28-year career at Sandia, she has worked as a principal investigator on a diverse technical portfolio. Currently, Cecelia leads the program's Southeast Asia biorisk engagement initiatives, and conducts analyses to support the US Government's biothreat decision-making. She also actively participates in many of the program's laboratory biorisk management training efforts. Previously, Cecelia was part of a Sandia team that developed an agriculture and food vulnerability assessment tool for FDA and USDA. She is also certified in Hazardous Waste Management and has worked on projects with the Russian Scientific Technology Center of the Mining and Chemical Combine on a number of technology demonstrations aimed at transitioning some Russian technologies to the USA. Cecelia earned her PhD from Texas A & M University, researching the interaction of a Rotavirus enterotoxic protein with cellular caveolin.

Susan Boggs, PhD

Laura Jones, MS

Laura Jones is a Member of the Technical Staff in the International Biological Threat Reduction Program at Sandia National Laboratories. Laura leads the program's development of core laboratory biorisk documents, including manuals and standard operating procedures. She also leads biorisk engagement efforts in Gulf Cooperation Council member countries and Malaysia. Additionally, she is a key contributor to biothreat identification, prevention, and response efforts in Africa. Prior to working at Sandia, Laura worked in water security and led the development of guidance for large-scale decontamination of water contaminated with biothreats agents. Laura received Bachelor's and Master's degrees in Microbiology from New Mexico State University. Her Master's thesis focused on rapid detection of biothreat agents in large volumes of water.

Course Agenda

Day 1 –Sunday, June 10th

	All Groups
Morning 8:30-12:30	**Introductions and Course Overview**
	Pre-Assessment Test
	Break
	Orientation to Biorisk Management (GBRMC)
12:30-1:30	**Lunch**
Afternoon 12:30-4:30	**Biorisk Assessment, Risk Characterization and Evaluation**
	Break
	Risk Assessment continued
4:30-5:30	**Hypothetical Facility / Case Study and Recap**
5:30	**End of the Day**

Day 2 –Monday, June 11th

	All Groups	
8:30-9:00	**Risk Assessment Review**	
Morning 9:00-12:30	**Group 1 Laboratory – Hands on activities to assess risk of specific laboratory procedures**	**Group 2 – Good Laboratory Practices**
	Break	**Break**
	Group 1 – Good Laboratory Practices	**Group 2 - Laboratory – Hands on activities to assess risk of specific laboratory procedures**
12:30-1:30	**Lunch**	**Lunch**
Afternoon 1:30-4:30	**Personal Protective Equipment**	
	Break	**Break**
	Designing and testing PPE donning and doffing	
4:30-5:30	**Case Study and Recap**	
5:30	**End of the Day**	
6:30	**Vans Leave Hotel for Dinner in Traditional Jordanian Restaurant**	

Day 3 – Tuesday, June 12th

	All Groups	
8:30-9:00	**Personal Protective Equipment and Good Laboratory Practices Review**	
Morning 9:00-12:30	**Group 1 - Waste Handling**	**Group 2 – Laboratory – Biosafety Cabinets, Equipment and Air Flow**
	Break	**Break**
	Group 1 – Decontamination, Sterilization and Disinfection	**Group 2 - Laboratory**
12:30-1:30	**Lunch**	**Lunch**
Afternoon 1:30-4:30	**Group 1 – Laboratory – Biosafety Cabinets, Equipment and Air Flow**	**Group 2 – Waste Handling**
	Break	**Break**
	Group 1 - Laboratory	**Group 2 – Decontamination, Sterilization and Disinfection**
4:30-5:30	**Case Study and Recap**	
5:30	**End of the Day**	

Day 4 – Wednesday, June 13th

	All Groups
8:30-9:00	**Waste Handling and Decontamination Review**
Morning 9:00-12:30	**Introduction to Laboratory Biosecurity**
	Break
	Laboratory Biosecurity Continued
12:30-1:30	**Lunch**
Afternoon 12:30-4:30	**Emergency and Incident Planning and Response**
	Break
	Incident Response - continued
4:30-5:30	**Case Study and Recap**
5:30	**End of the Day**

Day 5 – Thursday, June 14th

	All Groups
8:30-9:00	**Incident Response and Laboratory Biosecurity Review**
Morning 9:00-12:30	**Biorisk Management System Performance**
	Break
	BRM Performance - continued
12:30-1:30	**Lunch**
Afternoon 12:30-4:30	**Review of the workshop and Final Exam**
	Break
	Presentation of Case Studies
4:30	**End of the Day**

Day 6 and 7 – Friday and Saturday, June 15th/16th – Off Days

No formally organized activities these days.

CPHL Staff Day 8 - Shipping Infectious Substances – Sunday, June 17th

	Lab directors and technicians
Morning 8:30-12:30	**Introduction to Shipping Infectious Substances and Diagnostic Specimens**
	Determine Requirements to Ship Samples
	Break
	Classifying Shipments
12:30-1:30	**Lunch**
Afternoon 1:30-5:30	**Packaging**
	Break
	Packaging Continued
	Case study - Packaging
5:30	**End of the Day**

CPHL Staff Day 9 - Shipping Infectious Substances – Monday, June 18th

	Lab directors and technicians
Morning 8:30-12:30	**Marking and Labeling**
	Documentation
	Break
	Program Management – Developing a shipping program
12:30-1:30	**Lunch**
Afternoon 1:30-5:30	**Review**
	Break
	Final Exam
	Certificate presentation
5:30	**End of the Day**

INMD Staff Day 8 - Biorisk Management Policy and Program – Sunday June 17th

	INMD Staff
Morning 8:30-12:30	**Identifying Legal Requirements that impact BRM**
	Break
	Identifying Legal Requirements that impact BRM
12:30-1:30	
Afternoon 1:30-5:30	**Writing and communicating BRM policy**
	Break
	Writing and communicating BRM policy
5:30	**End of the Day**

INMD Staff Day 9 - BRM Policy and Program – Monday June 18th

	INMD Staff
Morning 8:30-12:30	**Establishing and Communicating BRM roles, responsibilities, objectives, and goals**
	Break
	Establishing and Communicating BRM roles, responsibilities, objectives, and goals
12:30-1:30	**Lunch**
Afternoon 1:30-5:30	**Work Program Review and Approval**
	Break
	Work Program Review and Approval
5:30	**End of the Day**

Acronyms:

ABSL – Animal Biosafety Level
BBP – Blood Borne Pathogen
BSC – Biosafety Cabinet
BSL – Biosafety Level
BWC – Biological and Toxins Weapons Convention
CDC – US Centers for Disease Control and Prevention
CWA – CEN (European Committee for Standardization) Workshop Agreement
ELISA – Enzyme-linked-immunosorbent serologic assay
FBC – Facility Biorisk Committee
GLP – Good Laboratory Practices
HEPA – High Efficiency Particulate Air
HPAI – Highly Pathogenic Avian Influenza
IACUC – Institutional Animal Care and Use Committee
IBC – Institutional Biosafety Committee
IBTR – International Biological Threat Reduction
MDR – Multi-drug resistant
PCR – Polymerase Chain Reaction
PPE – Personal Protective Equipment
RG – Risk Group
RT-PCR – Reverse transcriptase polymerase chain reaction
SNL – Sandia National Laboratories
UN – United Nations
WHO – World Health Organization

Glossary:

Aerosol – A substance consisting of very fine particles of a liquid or solid suspended in a gas.

Agarose – A polysaccharide obtained from agar that is the most widely used medium for gel electrophoresis procedures.

Aliquot – Comprising a known fraction of a whole and constituting a sample.

Animal Biosafety Level – Combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity in an area where animals are present.

Assay – A quantitative determination of the amount of a given substance in a particular sample.

Autoclave – An apparatus in which steam under pressure effects sterilization.

Biohazard – A biological agent, such as an infectious microorganism, that constitutes a threat to humans or to the environment.

Biosafety Cabinet – A device enclosed (except for necessary exhaust purposes) on three sides and top and bottom, designed to draw air inward by means of mechanical ventilation, operated with insertion of only the hands and arms of the user, and in which virulent pathogens are used. Biosafety Cabinets are classified as: Class I, Class II and Class III, each providing different levels of protection.

Biosafety Level – A combination of work practices, primary containment devices and construction technology to reduce the risk of exposure resulting in laboratory acquired infection or release of a microbe to the environment.

Biosafety Level 1 – Represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing. *Agents that can be worked with at BSL1:* Microorganisms not known to cause disease in healthy adult humans

Biosafety Level 2 – Similar to Biosafety Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. *Agents that can be worked with at BSL2:* Indigenous, moderate-risk agents associated with human disease of varying severity

Biosafety Level 3 – All procedures in this laboratory involving the manipulation of infectious materials are conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. The laboratory has special engineering and design features. *Agents that can be worked with at BSL3:* Indigenous or exotic agents where the potential for infection by aerosol exists and disease may have serious-to-lethal consequences

Biosafety Level 4 – The facility is either in a separate building or in a controlled area within a building, which is completely isolated from all other areas of the building. A specific facility operations manual is prepared or adopted. Within work areas of the facility, all activities are confined to Class III biological safety cabinets, or Class II biological safety cabinets used with one-piece positive pressure personnel suits ventilated by a life support system. The Biosafety Level 4 laboratory has special engineering and design features to prevent microorganisms from being disseminated into the environment. *Agents that can be worked with at BSL4:* Dangerous and exotic agents that pose a high risk of life threatening disease

Blood-borne Pathogen – Micro-organisms that are present in human/ primate blood, tissues or fluids that can cause disease in humans. These pathogens include (but are not limited to) hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Carrier – A person, animal, or plant that serves as a host for a pathogen and can transmit it to others, but is immune to it.

Centrifuge – Equipment that separates substances of different densities in a sample by rotation at very high speed, forcing the substance to be displaced outward, sometimes through a series of filters or gratings. Substances with greater density are displaced from the center more than ones that are less dense.

Containment – The control of biohazards through practices & procedures, primary barriers, and secondary barriers.

Decontamination – Removing disease-causing organisms from contaminated articles or surfaces.

Disinfection – Selective elimination of certain undesirable microorganisms in order to prevent their transmission.

Doffing – To remove or take off.

Donning – To put on or dress in.

Endemic – Relating to a disease consistently present in a population in a particular locality.

Enzootic – Relating to a disease consistently present in a population of animals in a particular locality.

Epidemic – Disease occurring in larger numbers than usual or in excess of normal expectancy.

Epizootic – A disease affecting many animals at the same time, and spreading from animal to animal in a particular locality.

Fomite – An inanimate object or substance that is capable of transmitting infectious organisms from one individual to another.

Germicide – Any antimicrobial chemical agent used to kill disease-causing organisms.

Homogenize – To form by blending unlike elements; to make uniform or similar, as in composition or function.

Host – The animal or plant on which or in which another organism lives.

Incident – abnormal or unplanned event or conditions that adversely affect or potentially affect safety or security.

Infection – Invasion by and multiplication of pathogenic microorganisms in body tissues, which may produce subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms.

Laboratory biosafety – A set of preventive measures designed to reduce the risk of accidental exposure to or release of a biological hazard

Laboratory biosecurity – A set of preventive measures designed to reduce the risk of intentional removal (theft) of a valuable biological material

Latent infection – An infectious agent or disease that remains in an inactive or hidden phase; dormant.

Lyophilizer – Equipment used for freeze-drying.

Mucous membrane – Any of the membranes lining the passages of the body, such as the respiratory and digestive tracts, that open to the outside. Cells in the mucous membranes secrete mucus, which lubricates the membranes and protects against infection.

Pathogen – An agent that causes infection or disease, such as a bacterium or protozoan, or a virus.

Polymerase Chain Reaction – A technique for amplifying DNA sequences in vitro by separating the DNA into two strands and incubating it with oligonucleotide primers and DNA polymerase.

Reservoir – An organism or a population that directly or indirectly transmits a pathogen while being virtually immune to its effects.

Risk – The function of the likelihood and consequences of an adverse event; biorisks involve biological agents or their products.

Risk assessment – A systematic, structured process for analysis and determination of risks

Risk Group – Infectious agents are categorized in risk groups based on their relative risk. Risk group classifications are primarily used in the research environment as part of a comprehensive biosafety risk assessment.

Risk Group 1 – No or low individual and community risk, A microorganism unlikely to cause human or animal disease.

Risk Group 2 – Moderate individual risk, low community risk. Pathogen causes human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. May cause serious infection but effective treatments and preventive measures are available. Risk of spread is limited.

Risk Group 3 – High individual risk, low community risk. Pathogen usually causes serious human or animal disease but does not ordinarily spread to others. Effective treatment and preventive measures are available.

Risk Group 4 – High individual and community risk. A pathogen causes serious human or animal disease; readily transmitted from one individual to another. Effective treatment and preventive measures are usually not available.

Rotor – The device used to hold tubes during centrifugation.

Reverse transcriptase polymerase chain reaction – PCR process by which copies of DNA are generated from RNA.

Valuable Biological Material – A biological agent that has use either in research or for malicious purposes, commonly both

Vector -
a. An insect or other organism that transmits a pathogenic fungus, virus, bacterium, etc.

b. Any agent that acts as a carrier or transporter, as a virus or plasmid that conveys a genetically engineered DNA segment into a host cell.

Vortexer – A device used to mix, by means of a rapid whirling or circular motion.

Zoonotic Diseases – A disease of animals, such as rabies or psittacosis, which can be transmitted to humans.

Learning Objectives

By the end of the course, participants should be able to meet the following objectives:

1. Understand the risk assessment process

    a. Define risk
    b. List agent factors to be considered during risk assessment
    c. Explain how biosafety and biosecurity are mutually supportive
    d. Perform a risk assessment

2. Understand the risk mitigation process

    a. Use the results of a risk assessment to select appropriate risk mitigation measures
    b. Select proper PPE based on agent characteristics and work being performed
    c. Demonstrate proper laboratory entry and exit procedures, including donning and doffing of PPE
    d. Identify techniques to minimize aerosol production
    e. Set up an inventory system for biological agents
    f. Describe how directional airflow helps mitigate risks
    g. Describe key considerations for the selecting a type of biosafety cabinet
    h. Properly set up a biosafety cabinet
    i. Recognize the different types of infectious waste
    j. Verify that an autoclave operates correctly
    k. Describe how to respond properly to incidents
    l. Identify proper mechanisms for authorizing and controlling access

3. Understand the risk management process

    a. List elements of a program management system
    b. Describe the roles and responsibilities of the biorisk officer
    c. List types of documentation and training necessary for a successful program
    d. Outline solutions to potential conflicts between biosafety and biosecurity

Biorisks

What are the risks of working in a laboratory with biological material?

Work in your group to identify some of these risks

1.

2.

3.

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- ***Laboratory biosafety****: containment principles, technologies, and practices implemented to prevent unintentional exposure to pathogens and toxins, or their unintentional release*[1]
- ***Laboratory biosecurity****: protection, control and accountability for valuable biological materials within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release.* [2]

[1] *Laboratory biosafety manual, Third edition (World Health Organization, 2004)*

[2] *Biorisk management - Laboratory biosecurity guidance (World Health Organization, 2006)*

biorisk (adapted from ISO/IEC Guide 51:1999)

combination of the probability of occurrence of harm and the severity of that harm where the source of harm is a biological agent or toxin

NOTE: The source of harm may be an unintentional exposure, accidental release or loss, theft, misuse, diversion, unauthorized access or intentional unauthorized release.

Biorisk encompasses both biosafety and biosecurity

In your group, answer the following questions

How do you identify risks?

How do you manage risks?

How do you know your management strategy is working?

☣ Biorisk Assessment

- Process of identifying the hazards and evaluating the risks associated with biological agents and toxins, taking into account the adequacy of any existing controls, and deciding whether or not the risks are acceptable

☣ Biorisk Mitigation

- Actions and control measures that are put into place to reduce or eliminate the risks associated with biological agents and toxins

☣ Performance

- The implementation of the entire biorisk management system, including evaluating and ensuring that the system is working the way it was designed. Another aspect of performance is the process of continually improving the system.

Biorisk Management =
Assessment, Mitigation, Performance

Biorisk Assessment

Learning Objectives

- Be able to define risk.
- Be able to explain the dependence of likelihood and consequences on the risk assessment.
- Be able to describe the process of model development.
- Be able to describe the advantages of a robust structured risk assessment process.

Key Principles

- A biosafety and biosecurity risk assessment allows a laboratory to determine the relative level of risk its different activities pose, and helps guide risk mitigation decisions so these are targeted to the most important risk.
- Risk Characterization is the process of identifying the factors that contribute to risk and determining the likelihood and consequences that contribute to risk.
- Complete and thorough analysis of the different hazards, threats and situations that can affect risk will increase the robustness of the risk characterization process.
- Risk Evaluation is a crucial intermediary step between Risk Characterization and taking active steps towards mitigating risk and is the process of determining whether a particular risk is in fact acceptable or not to a facility or institution.

A **biological risk assessment** is an analytical procedure designed to characterize biological risks in a facility, laboratory or unit within it, or other type of operation dealing with potential pathogens or toxins.

Generally, we can classify biological risk assessments into two types:

- **Biosafety risk assessment**
- **Biosecurity risk assessment**

Why is doing a risk assessment important?

Laboratory biosafety manual, Third edition (World Health Organization, 2004) defines the following:

- **Laboratory biosafety**: containment principles, technologies, and practices implemented to **prevent** **unintentional** exposure to pathogens and toxins, or their unintentional release
- **Laboratory biosecurity**: institutional and personal security measures designed to **prevent** the loss, theft, misuse, diversion, or **intentional** release of pathogens and toxins

What is Risk? Develop a definition of "Risk" and list some of the key components of "Risk"

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2.

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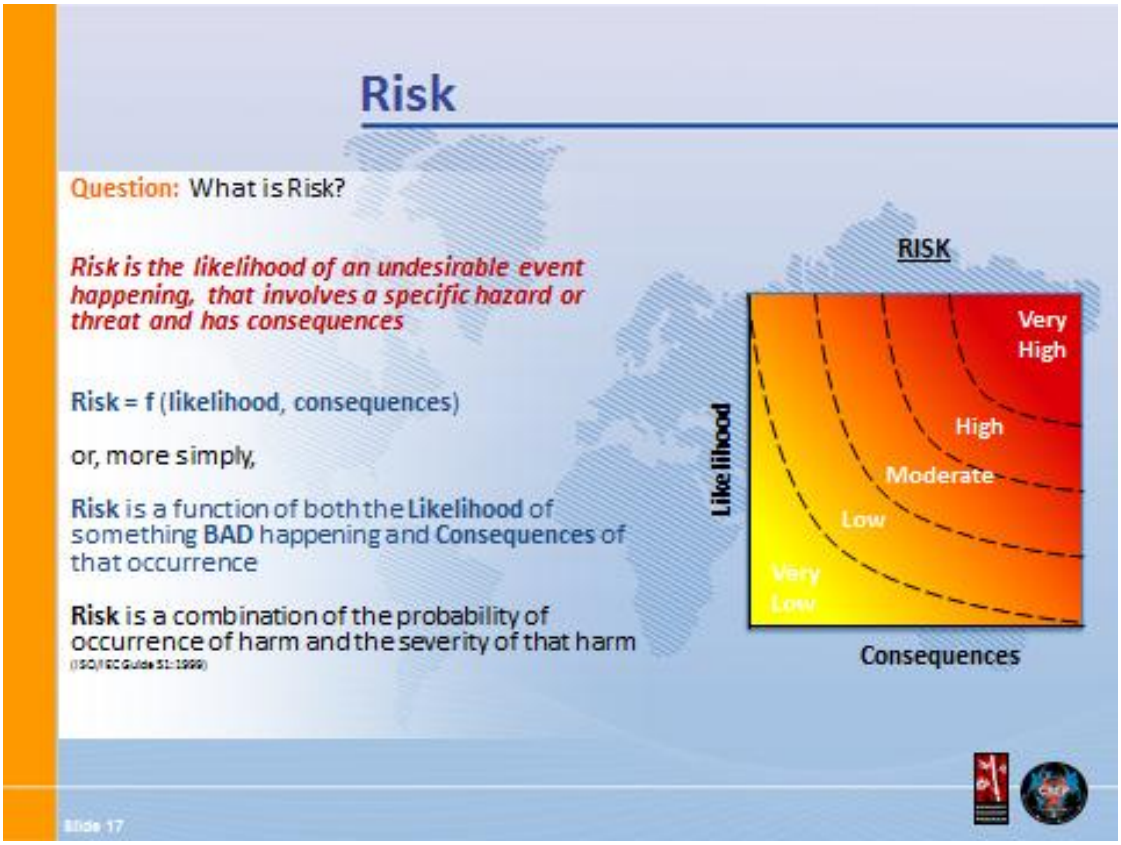
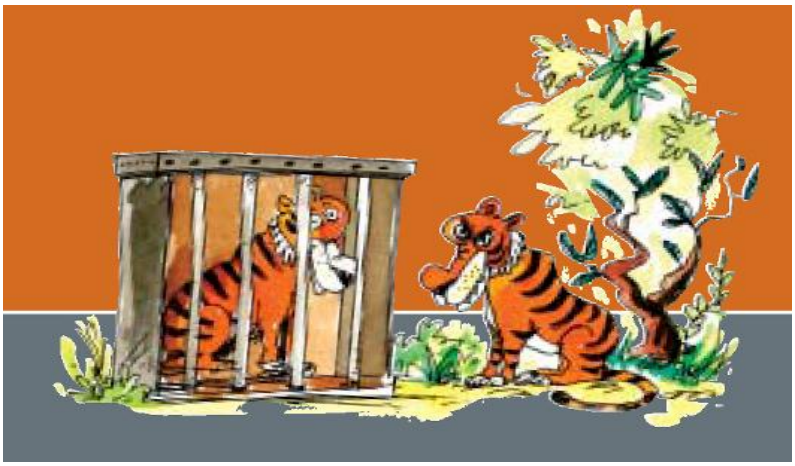
What is Risk?



- A **hazard** is a source that has a potential for causing harm
- A **threat** is a person who has intent and/or ability to cause harm
- A risk can be based on either a hazard and/or a threat
- The term **hazard** is used in the **biosafety** context, and threat is used in the **biosecurity** context.

What is the risk of being attacked by a Tiger?



Work in your group to identify some of these factors that would help you determine this risk.

1.

2.

3.

4.

5.

6.

7.

Risk Governance Strategy

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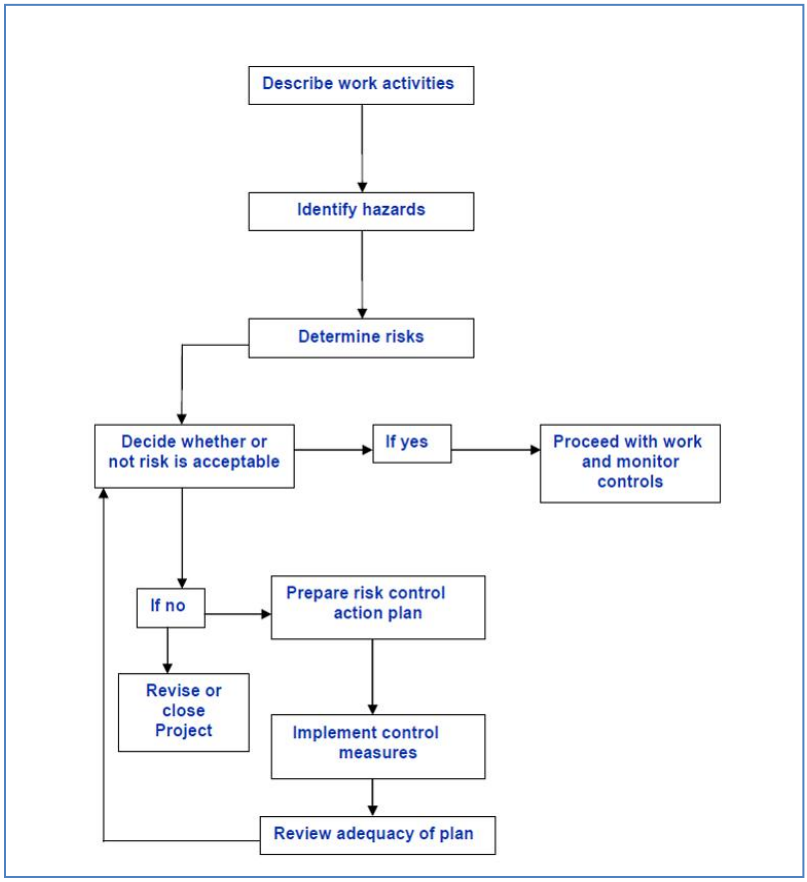
The first stage in the risk management process is to identify all hazards that are relevant for biorisk. It is useful to involve the whole work team in this process and to use inputs from organizational experts on safety and risk management.



A hazard may be a physical situation (e.g. a fire or explosion), an activity (e.g. pipetting) or a material (in this case the principal hazard is most likely to be a biological agent or toxin, but others will include chemicals and asphyxiating gases such as nitrogen). The essence of a hazard is that it has the potential for causing harm, regardless of how likely or unlikely such an occurrence might be.

Biological hazards should be identified and assessed in relation to their potential damage to humans, animals, and the environment. Where hazardous materials are classified into hazard or risk groups based on international and/or foreign country classification schemes local diverging needs and constraints should be considered.

A hazard identification exercise should use information including:

a) group experience and knowledge;
b) external or specialized expertise not found in the facility;
c) results of previous assessments;
d) surveys of previous accidents/incidents;
e) hazardous materials data;
f) information on hazardous organisms;
g) guidelines and codes of practice;
h) facility drawings;
i) SOPs, manuals, etc.;
j) process maps.

Group Activity Step 1: Think about the tiger scenario, and the broad categories that you came up with. What are some of the broad **categories** of factors that you need to look into to conduct a **biosafety risk assessment**? or a **biosecurity risk assessment**?

Categories of factors to consider when conducting a laboratory **biosafety** risk assessment:

1.

2.

3.

4.

5.

Categories of factors to consider when conducting a laboratory **biosecurity** risk assessment:

1.

2.

3.

4.

5.

For one of the identified categories of risk factors, define the questions you need to answer to conduct a risk assessment. What are the key questions you need to answer to define the **likelihood** for exposure to a biohazardous agent in the lab:

Likelihood

Question	Best Case Scenario	Worst Case Scenario
1. Are sharps being used?	No sharps in use	Sharps used without training, controls, or appropriate PPE…
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

For one of the identified categories of risk factors, define the questions you need to answer to conduct a risk assessment. What are the key questions you need to answer to define the **consequences** of exposure to a biohazardous agent in the lab:

Consequences

Question	Best Case Scenario	Worst Case Scenario
1. What is the mortality rate of the agent?	No mortality	High rate of mortality (over 90%)
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		

Risk Characterization

Risk Characterization is the actual process of determining the **likelihood** and **consequences** of a particular risk within a **Risk Assessment**.

Please recall that risk is associated with a **particular adverse event**. We can only determine the **likelihood** and **consequences** of a very clearly defined risk.

As a reminder, part of this process is the identification of the appropriate **hazard** or **threat**.

The **hazard** or **threat** is the **source** or **causative agent** of a particular **risk**.

The term **hazard** is used in the **biosafety** context, and **threat** is used in the **biosecurity** context.

For **Biosafety Risk**, the **hazard** is the biological material worked with in the lab.

Characterizing the material allows one to determine important parameters for **likelihood** and **consequences**, such as **route of exposure**, **infectious dose**, **incubation time**, **morbidity**, **mortality**, **communicability**, and others.

For **Biosecurity Risk**, the **threat** is the potential adversary who is interested in the biological materials.

Characterizing potential adversaries allows one to determine important parameters for **likelihood** and **consequences**, such **means**, **motives**, and **opportunity**.

Risk characterization scenarios:

Scenario 1:

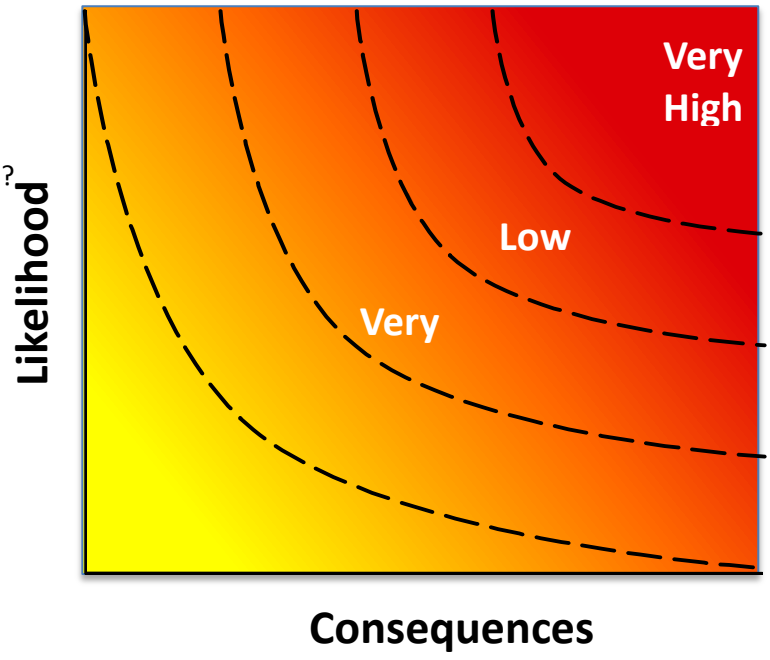
A lab worker has just prepared a culture of *Mycobacterium tuberculosis* in order to conduct a drug sensitivity test. He is wearing gloves, a surgical mask, and lab coat, and is using a Biosafety Cabinet which has not been certified in three years.

What is the **hazard** in this scenario?

What is the **likelihood** of exposure?

What are the **consequences** of exposure?

What are some factors that should be considered?



Scenario 2:

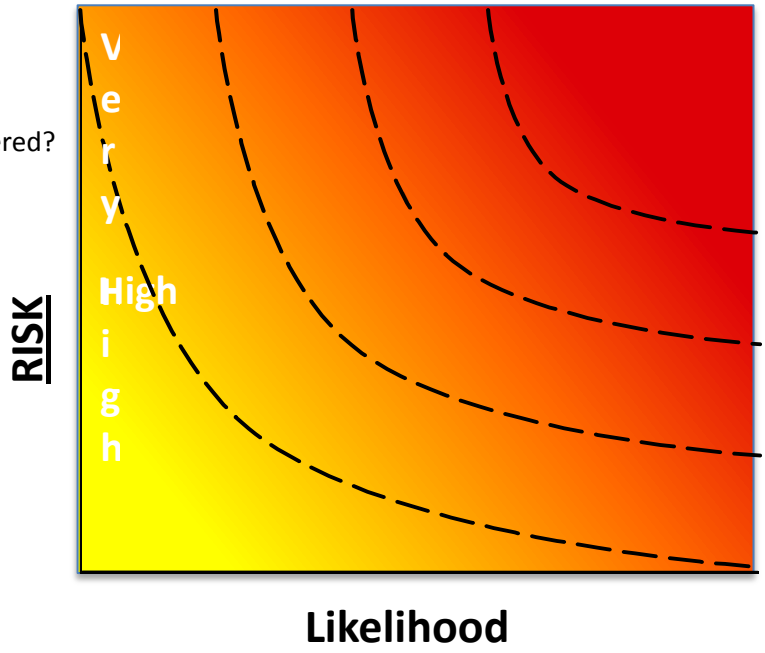
The lab worker discovers his culture of *Mycobacterium tuberculosis* is Extremely Drug Resistant (XDR). Meanwhile, a local animal rights group has threatened to disrupt laboratory operations and generate publicity by breaking in and stealing agents. They believe taking samples of XDR will generate the most publicity.

What is the **threat** in this scenario?

What is the **likelihood** of a theft?

What are the **consequences** of a theft?

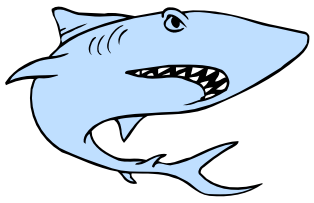
What are some factors that should be considered?



A **hazard** or **threat** cannot in itself pose a **risk** without a specific **situation**.

Conversely, a **situation** also does not represent a **risk** without a **hazard** or **threat**.

Both a changing **hazard** or **threat**, and a changing **situation** will independently alter the scenario being assessed, and thus change the **risk**.



Relative Risk

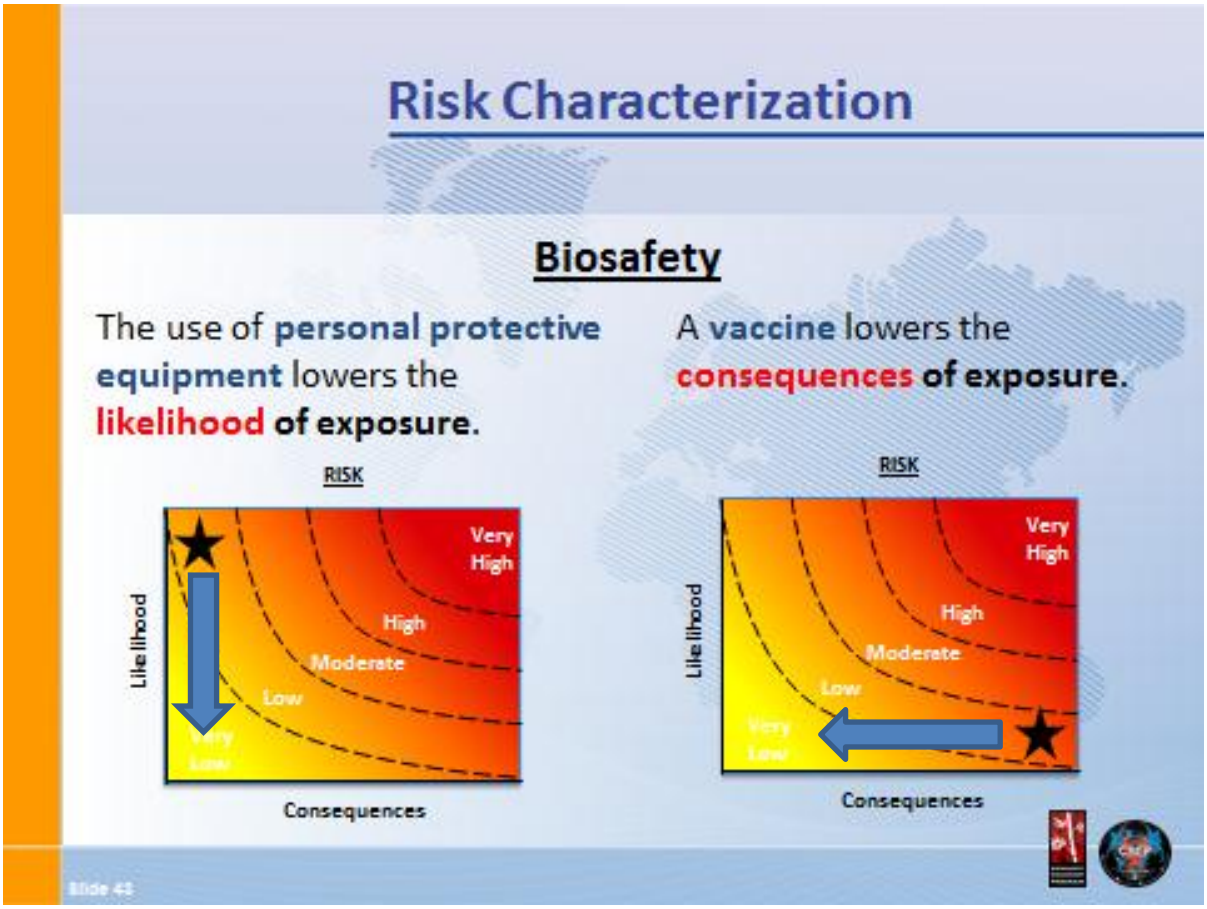
Another consideration in characterizing risk is the concept of **relative risk**.

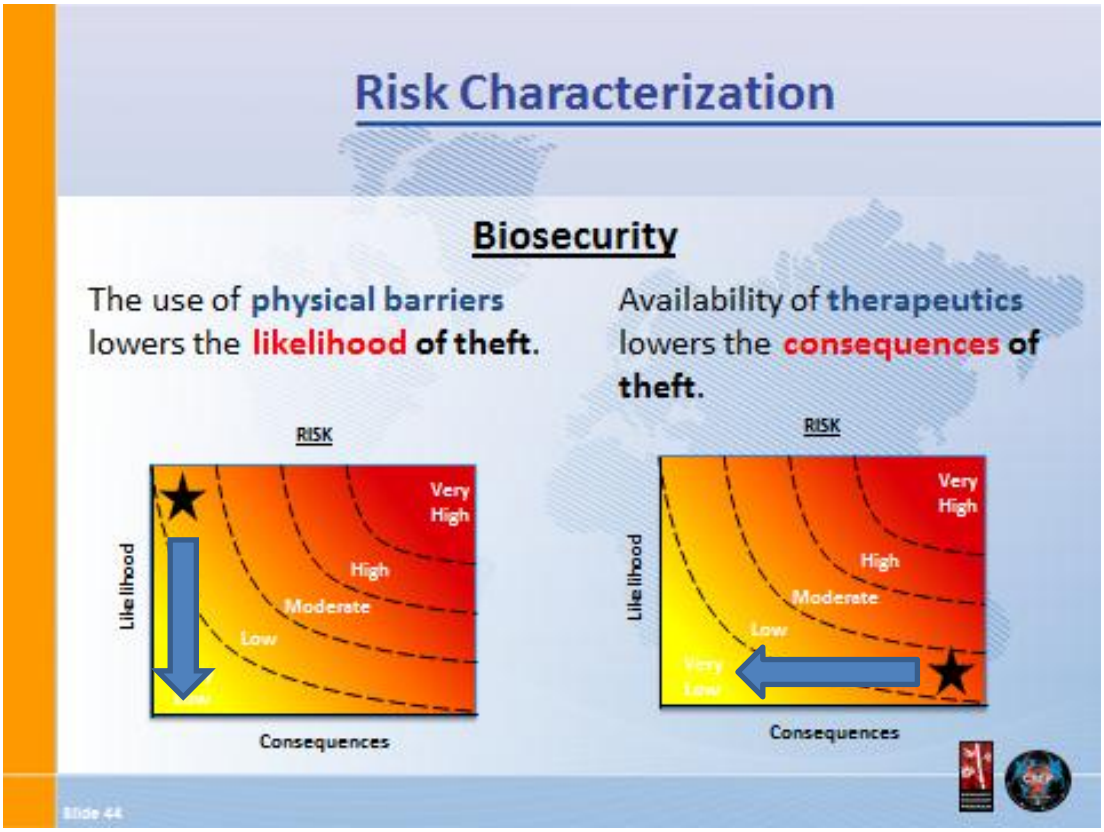
Relative risk is the value of one particular risk (in terms of likelihood and consequences) in the context of another risk. It helps give meaning to risk.

For example:

Dependent on certain situation, or the PPE used, the risk of aerosol exposure may be higher than the risk of percutaneous exposure.

Risk is relative because it will change depending on the situation and the factors that go into determining the risk, themselves, are not absolute.





Understanding the **relative risk** associated with **different situations** or by implementing **different risk mitigation strategies** is important for the overall process of **characterizing risk**.

Naturally, each **facility**, **laboratory**, and **situation** will have its own unique set of **factors** that will be particularly relevant to fully **characterize risk**.

It is important that the **Risk Characterization** process be as **robust** as possible.

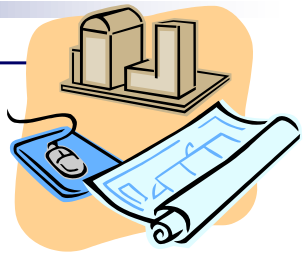
Comparability is the ability trust the accuracy of *differences* between assessments, due to similarities in their bases, assumptions, procedures and protocols.

Repeatability is the ability to conduct the same process in the same way for the same hazard or threat and situation over a period of time, or for different hazards, threats, and situations at the *same* time.

A **robust risk characterization** process will generate similar results when assessing similar hazards or threats in similar situations, no matter who is following the methodology, where the characterization is being conducted, and when it's occurring.

A **robust risk characterization** makes a **risk assessment robust**.

BioRAM

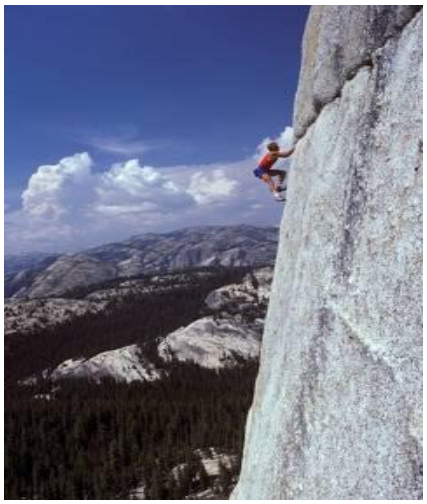


- **Bio**safety **R**isk **A**ssessment **M**odel
- **Bio**security **R**isk **A**ssessment **M**odel
- Both have relied extensively on external experts from the international community
- Available through the following URL:

http://www.biosecurity.sandia.gov/BioRAM/

What are some of the benefits of a robust structured risk assessment process?

Risk Evaluation



Risk Evaluation is a crucial intermediary step between Risk Characterization and taking active steps towards mitigating risk.

Risk Evaluation is the process of determining, subjectively, whether a risk is **high** or **low**, and whether it's **acceptable** or not.

Unfortunately, there is **no systematic way** of evaluating risk and determining risk acceptability. This will depend on the perceptions of **individuals**, **institutions**, and the **community**.

What are some considerations when determining if a risk is acceptable?

1.

2.

3.

4.

5.

6.

7.

Individual Reflection

When you think about conducting a risk assessment, what are some of the key messages you will take home and use?

Biorisk Mitigation

Actions and control measures that are put into place to reduce or eliminate the risks associated with biological agents and toxins.

Learning Objectives

- Understand the role of mitigation in the AMP model for biorisk management
- Appreciate that mitigation must be based on a thorough risk assessment
- List the categories of control measures and describe the hierarchy of controls
- Understand the advantages and limitations of each type of biorisk mitigation measure
- Be prepared to learn more details about specific mitigation strategies

Why is it important to conduct a Risk Assessment prior to implementing mitigation controls?

What outcomes would you expect when mitigation is based on a thorough risk assessment?

What would you expect if mitigation is implemented without conducting a risk assessment?

Group Exercise

Using your case study, identify at least ten different risk mitigation measures. Measures should address both safety and security.

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

- **Engineering Controls:** Physical changes to work stations, equipment, materials, production facilities, or any other relevant aspect of the work environment that reduce or prevent exposure to hazards
- **Administrative Controls:** Policies, standards and guidelines used to control risks
- **Practices and Procedures:** Processes and activities that have been shown in practice to be effective in reducing risks
- **Personal Protective Equipment:** Devices worn by the worker to protect against hazards in the laboratory

Mitigation Control Measures

- Elimination or Substitution: Removing the hazard, not doing the work, or replacing the hazard with something with less risk

International BIOLOGICAL THREAT REDUCTION

Control Measures – Advantages/Disadvantages

Control Measure	Advantages	Disadvantages
Elimination/Substitution		
Engineering		
Administrative		
Practices/Procedures		
PPE		

Laboratory Practices

Learning Objectives

- Be able to discuss Good Laboratory Practices, and explain why they are "good".
- Be able to explain the importance of following proper procedure. (ex. Regular Hand washing versus Proper Hand washing)

What are good laboratory practices?

In your group, list several good laboratory practices. Write these practices on your flip chart and be prepared to discuss them with the class.

For your assigned laboratory practice or practices, answer the following questions:

- Why are they good practices?

- Why do them?

- Who/what are these practices protecting and from what?

- How can you insure people are following them?

Which good laboratory practices (use your list or the master list for guidance) can be enforced though which type of mitigation measure:

Elimination / substitution?	Administrative controls?	Engineering Controls?

Bad Laboratory Practices?

Look at the examples presented and identify the practice that is lacking.

Example 1:

Army: 3 vials of virus samples missing from Maryland facility

Story Highlights

- Venezuelan Equine Encephalitis samples missing from Army facility
- Virus sickens horses, can be spread to humans through mosquitoes
- Investigators say vials may have been in freezer that was destroyed
- So far, investigators have found no evidence of criminal activity

From Larry Shaughnessy
CNN Pentagon Producer

WASHINGTON (CNN) -- Missing vials of a potentially dangerous virus have prompted an Army investigation into the disappearance from a lab in Maryland. The Army's Criminal Investigation Command agents have been visiting Fort Detrick in Frederick, Maryland, to investigate the disappearance of the vials. Christopher Grey, spokesman for the command, said this latest investigation has found "no evidence of criminal activity."

The vials contained samples of Venezuelan Equine Encephalitis, a virus that sickens horses and can be spread to humans by mosquitoes. In 97 percent of cases, humans with the virus suffer flu-like symptoms, but it can be deadly in about 1 out of 100 cases, according to Caree Vander Linden, a spokeswoman for the Army's Medical Research Institute of Infectious Diseases. There is an effective vaccine for the disease and there hasn't been an outbreak in the United States since 1971.

The vials had been at the research institute's facility at Fort Detrick, home of the Army's top biological research facility, for more than a decade. The three missing vials were among thousands of vials that were under the control of a senior scientist who retired in 2004. When another Fort Detrick scientist recently inventoried the retired scientist's biological samples, he discovered that the three vials of the virus were missing. The original scientist's records about his vials dated back to the days of paper-and-pen inventories.

During the investigation, the retired scientist and another former Fort Detrick researcher cooperated with investigating agents and, according to Vander Linden, they came back to the facility to help look for the vials.
Vander Linden said the investigators know that several years ago an entire freezer full of biological samples broke down and all the samples had to be safely destroyed. But a complete inventory of what was in the freezer was not done before the samples were destroyed. Vander Linden said there's a "strong possibility" the vials were in that freezer and destroyed, but that isn't known for sure.

This investigation comes two months after all research at the research institute facility at Fort Detrick was halted for a complete computer-based inventory of all disease samples at the fort. That inventory is expected to be complete before summer and may help solve the mystery of the three missing vials, officials said.

The Army investigation is in its final stages and is expected to be closed soon.

EXAMPLE	What is lacking from the example?
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	

Summary

What are some of the key considerations of good laboratory practices and why are they "good"?

Individual Reflection

When you return to your laboratory, what are some laboratory practices you are considering changing? Adding, removing, or updating to ensure better compliance?

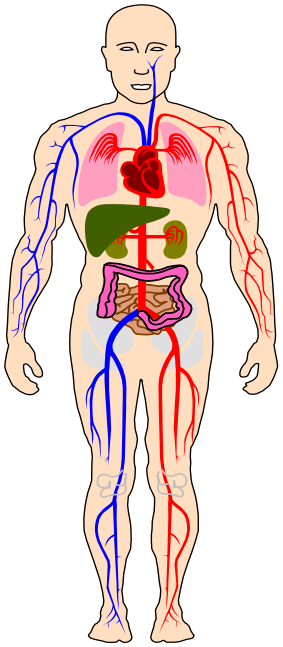
Personnel Protective Equipment (PPE)

Learning Objectives

- Be able to describe the differences between various routes of exposure.
- Be able to compare and contrast the utility of different articles of PPE.
- Be able to explain the principles of donning and doffing.

Why use personal protective equipment?

What are the possible routes of exposure that should be considered when selecting PPE?



PPE Selection

Examine the collection of PPE examples you have been provided.

Example 1:

What route of exposure do they protect?

What are the pros and cons of each example?

PPE EXAMPLE	Advantage	Disadvantage

What are some other considerations for each example?

Example 2:

What route of exposure do they protect?

What are the pros and cons of each example?

PPE EXAMPLE	Advantage	Disadvantage

What are some other considerations for each example?

Donning and Doffing

Is the order you put on your PPE important? Why? Discuss in your group if order matters and why.

Is the order you remove your PPE important? Why? Discuss in your group if order matters and why.

What are some key considerations in creating and order for donning and doffing?

1.

2.

3.

4.

5.

6.

Be prepared to share your considerations and rational with the class.

Case Examples

1. Medical technologists are using blood cultures to diagnosis *Brucella melitensis* using commercial blood culturing systems.

2. Medical technologists are using clinical stool samples to look for diarrheal diseases, focusing on multi-drug resistant strains of *Shigella sonnei.* The technologists are culturing the bacteria, and upon identification of *Shigella* strains are conducting sensitivity tests looking for multi-drug resistant strains.

3. A bovine has been exhibiting signs of Foot and Mouth Disease, and a veterinarian has collected nasal swabs which have been sent to the laboratory for testing. Testing will be done using real-time polymerase chain reaction (RT-PCR).

4. A researcher is conducting drug sensitivity studies of tuberculosis (TB) positive human samples to determine if strains are multi-drug-resistant (MDR-TB) or extensively drug-resistant (XDR-TB). Studies are done in culture tubes.

Using your example, determine what PPE is the most appropriate and why. Create a donning and doffing order for the PPE you have selected. Identify where PPE is stored, disposed of and/or cleaned, are there any other considerations?

PPE Required: *(you may need more of less items of PPE for your example)*

1.

and why?

2.

and why?

3.

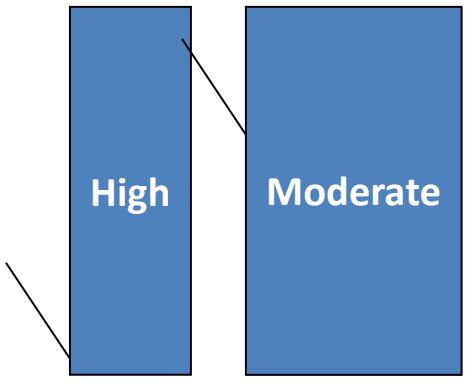
and why?

4.

and why?

Use the following laboratory in your design:

Where is it stored and disposed of and/or cleaned?



Create a donning and doffing order.

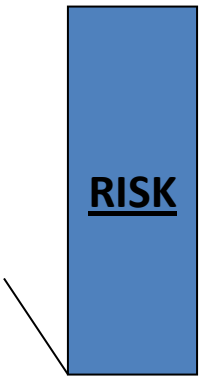
Donning **PPE and donning location**	**Doffing** **PPE and doffing location**
1.	1.
2.	2.
3.	3.
4.	4.
5.	5.
6.	6.

Test your donning and doffing order:

- Have one member of your team don the PPE in the order your team determined and enter the "lab"
- Once inside the "lab", the team member will be "contaminated" with a non-toxic material called Glow Germ. Glow Germ will foresee under UV or Black light to identify where it exists.
- The team member will remove the PPE in the doffing order determined

Did your PPE and ordering work?

Using your same example, redesign your donning an doffing order based upon this new laboratory design.



What are some of the key considerations when thinking about what PPE?

Individual Reflection

Are there any examples in your laboratory experiences where you have either used PPE that wasn't appropriate or failed to use the appropriate PPE?

Biological Waste Disposal

Learning Objectives

- Be familiar with the vocabulary applicable to biological waste
- Classify and segregate different types of biological waste, and select appropriate collection and storage methods
- Understand the factors that influence selection of treatment and disposal approaches and technologies
- Create a matrix of methods to treat and dispose different kinds of biological waste.

What kinds of waste exist in a typical laboratory? (Work in your group to identify several kinds of waste, write one answer per sticky note) – *think about your laboratory or others you have worked in or around*

Typical categories of biological waste:

- Solid waste (non-sharp)
- Sharps
- Pathological Waste
- Liquid Waste
- Mixed Waste

What are the risks posed from the waste for each category?

1.

2.

3.

4.

5.

Based upon those risks and waste categories you created, what will you process as infectious waste and how will you collect it in the laboratory, process it before or as leaving the laboratory, dispose of it, and how do you validate the process?

Waste Type	How is it segregated/collected inside laboratory?	How processed before or as leaving laboratory?	How will you dispose of the waste?	How will you validate the process?
Solid				
Sharps				
Pathological				
Liquid				
Mixed				

Decontamination, Sterilization, and Disinfection

Learning Objectives

- Discuss the differences between disinfection, decontamination, and sterilization.
- Discuss the various decontamination methods used for surface and area decontamination.
- Explain how validation of the decontamination procedure is conducted and be able to interpret the results.

Definitions

- ***Sterilization*** - act or process, physical or chemical, that destroys or eliminates all forms of life, especially microorganisms. The definition is categorical and absolute - an item either is sterile or is not.
- ***Disinfection*** - Generally less lethal process than sterilization. It is the elimination of nearly all recognized pathogenic micro-organisms but not necessarily all microbial forms (e.g., bacterial spores).
- ***Antiseptic*** - a substance that prevents or arrests the growth or action of microbes, either by inhibiting their activity or by destroying them

"septic" – containing disease causing organism, "anti" - remove

- ***Decontamination*** – A process to remove contamination. Decontamination renders an area, device, item, or material safe to handle, that is, reasonably free from a risk of disease transmission.

Typical Methods of Decontamination

- Chemical (e.g., bleach)
- Thermal (e.g., autoclave)
- Filtration (e.g., HEPA filter)
- Radiation (e.g., UV light)

Classes of Chemical Disinfectants

- Halogens (Chlorine, Iodophors)
- Aldehydes (Glutaraldehyde/Formaldehyde)
- Phenolics
- Alchohols
- Acids (Peracetic acid) & Alkalis (NaOH)
- Oxidizing Agents (Hydrogen peroxide)
- Quaternary Ammonium compounds
- Biguanidines (Chlorhexidine)

Group exercise

List the properties of the “ideal” chemical disinfectant

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

Group exercise

List the factors that might affect how well a chemical disinfectant will work

1. e.g. innate resistance to the disinfectant (see table below)

2.

3.

4.

5.

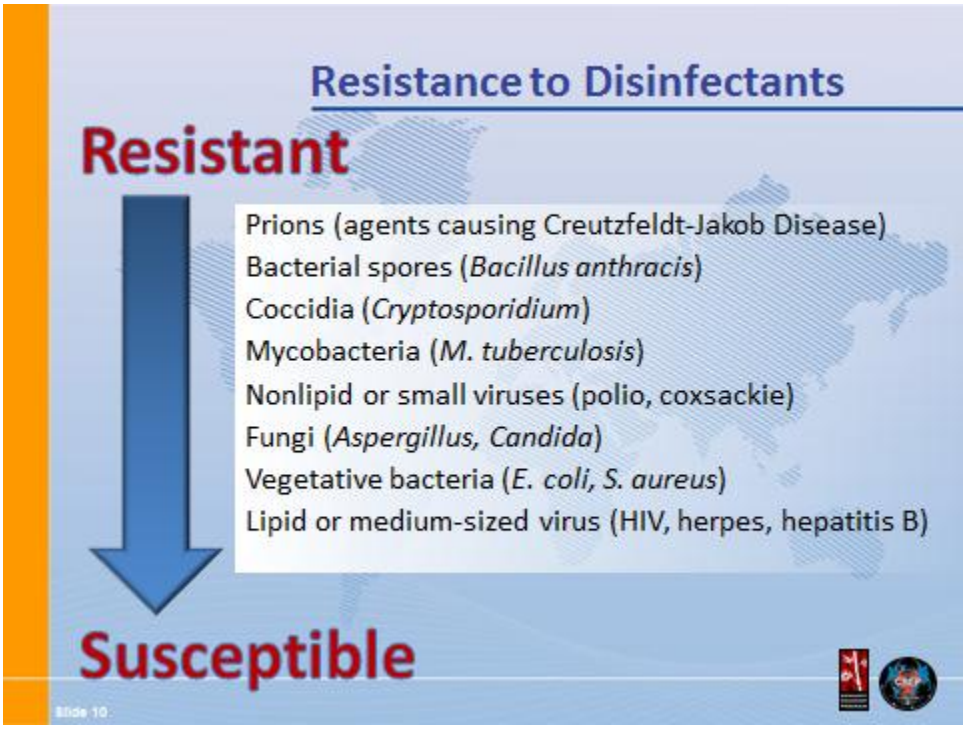
6.

7.

8.

9.

10.



Properties of Chemical Disinfectants

Product Name:

Mode of action:

Typical concentration used

Typical uses in the laboratory

Advantages or Benefits:

Disadvantages and limitations:

Choosing a Disinfectant

Please use the tables on the following pages to help you select a disinfectant each biological agent and situation in the table below. Briefly explain your choice. *(The tables provided are as they have been published in open source material and may contain errors, inconstancies, and gaps, these tables are representative of tables you may encounter in your work)*

Biological agent	**Decontaminating a work surface**	**Cleaning glassware**	**Explanatory notes**
E coli O157			
Vaccinia virus (enveloped virus)			
Mycobacterium bovis			
Clostridium botulinum spores			
Bluetongue virus (non-enveloped virus)			

TABLE 1

Practical Requirements							Inactivates			
			Contact Time (min.)							
Disinfectants **Type**	**Category**	**Use Dilution**	Lipovirus	Broad Spectrum	Temperature (^{o} C)	Rel. Humidity (%)	Vegetative Bacteria	Lipoviruses	Nonlipid Viruses	Bacterial Spores
Liquid	Quat Ammon.	0.1-2.0%	10	NE [A]			+	+		
	Phenolic	1.0-5.0%	10	NE			+	+	B	
	Chlorine	500 ppm c	10	30			+	+	+	+
	Iodophor	25-1600 ppm c	10	30			+	+	+	+
	Ethanol	70 – 85%	10	NE			+	+	B	
	Isopropanol	70 – 85%	10	NE			+	+	B	
	Formaldehyde	0.2-8.0%	10	30			+	+	+	+
	Glutaraldehyde	2%	10	30			+	+	+	+
Gas	Ethylene Oxide	8-23 g/**ft**^{3}	60	60	37	30	+	+	+	+
	Paraformaldehyde	0.3 g/ft^{3}	60	60	>23	>60	+	+	+	+

[A] NE-Not Effective

[B] Variable results dependent on virus

[C] Available Halogen

TABLE 2

Disinfectants Type	Disinfectants Category	Important Characteristics											
		EffectiveShelf Life(>1wk.[A])	Corrosive	Flam-mable	Explosion Potential	Residue	Inactivated by Organic Matter	Compatible for Optics[B]	Compatible for Electronics	Skin Irritant	Respiratory Irritant	Eye Irritant	Toxic[C]
Liquid	Quat Ammon	+					+	+		+		+	+
	Phenolic	+	+			+				+		+	+
	Chlorine		+			+	+			+	+	+	+
	Iodophor	+	+			+	+			+		+	+
	Ethanol	+		+								+	+
	Isopropanol	+		+								+	+
	Formaldehyde	+				+				+		+	+
	Glutaraldehyde	+				+		+		+		+	+
Gas	Ethylene Oxide	NA[D]		+[E]	+[E]			+	+	+	+	+	+
	Paraformaldehyde	NA		+[F]	+[F]			+	+	+	+	+	+

[A] Protected from light and air

[B] Usually compatible, but consider interferences from residues and effects on associated materials such as mounting adhesives

[C] Skin or mouth or both-refer to manufacturer's literature and /or Merck Index

[D] NA-Not Applicable

[E] Neither flammable nor explosive in 90%CO2 or fluorinated hydrocarbon, the usual form

[F] At concentrations of 7 to 73% by volume in air, solid-exposure to open flame

TABLE 3

Potential Application												
Disinfectants **Type**	**Category**	Work Sur-Faces	Dirty Glass-ware	Large Area Decon.	Air Handling Systems	Portable Equip. Surface Decon	Portable Equip. Penetrating Decon	Fixed Equip. Surface Decon	Fixed Equip. Penetrating Decon.	Optical and Electronic Instruments	Liquids for Discard	Books, Papers
Liquid	Quat. Ammon.	+	+			+		+				
	Phenolic	+	+			+		+				
	Chlorine	+	+			+		+			+	
	Iodophore	+	+			+		+				
	Ethanol	+	+			+		+				
	Isopropanol	+	+			+		+				
	Formaldehyde	+	+			+		+				
	Glutaraldehyde	+	+			+		+				
Gas	Ethylene Oxide						+			+		+
	Paraformaldehyde			+	+		+		+	+		

+ Very Positive response; + / — Less Positive Response; No entry denotes negative response or not applicable

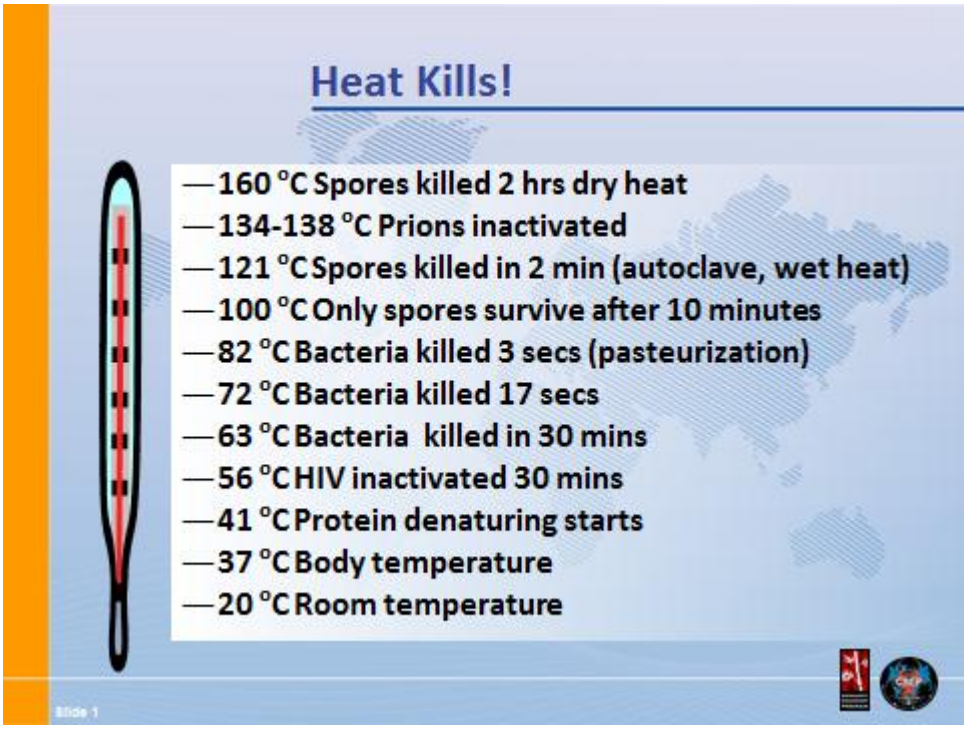
TABLE 4

Disinfectants		Vegetative Bacteria	LipoViruses	*M. tuberculosis*	Hydrophyllic Viruses	Bacterial Spores	**Examples of Proprietary Disinfectants**
Type	**Category**						
Liquid	Quat. Ammon.	+	+				A-33, CDQ, End-Bac, Hi-Tor, Mikro-Quat
	Phenolic	+	+	+	+ / —		Hil-Phene, Matar, Mikro-Bac, O-syl
	Chlorine	+	+	+	+	+ / —	Chloramine T, Clorox, Purex
	Iodophor	+	+	+	+ / —		Hy-Sine, Ioprep, Mikroklene, Wescodyne
	Ethanol	+	+	+	+ / —		Fisher, J.T.Baker, Mallinkrodt
	Isopropanol	+	+	+	+ / —		Fisher, J.T.Baker, Mallinkrodt, CVS,
	Formaldehyde	+	+	+	+	+ / —	Sterac
	Glutaraldehyde	+	+	+	+	+	Cidex
Gas	Ethylene Oxide	+	+	+	+	+	Carboxide, Cryoxide,Steroxide
	Paraformaldehyde	+	+	+	+	+	Fisher, J.T.Baker, Mallinkrodt

+ Very Positive response; + / — Less Positive Response; No entry denotes negative response or not applicable

These are a representative few of all the products available for disinfection. The listing or omission of a product neither rejects nor endorses use of the product.

Autoclaves



Principles of Autoclave sterilization

- Direct exposure to steam at the required temperature and pressure for a specific time
  - 121 °C – 123 °C
  - 15 psi; 1.05 kg/cm2
- Time required depends on the nature of the material to be sterilized. (Generally 1 hr for waste)
- Steam must directly contact all areas of the load (bags should be loosely gathered)
- If the steam can't penetrate a dry container, you have dry heat, which takes much longer to achieve kill.
- Add ~ 50 - 250 ml of water to bags prior to autoclaving to facilitate steam saturation

Autoclave Safety

- Follow manufacturers' guidelines
- Do not open pressurized chamber
- Avoid standing directly in front when opening
- Establish a preventative maintenance schedule and annual inspection by certified technician
- Wear appropriate PPE
- Careful – liquids are hot
- Open door slowly, allow steam to vent before opening fully
- Do not place sealed containers into autoclave
- Do not autoclave items containing solvents, volatiles, radioactive or corrosive chemicals
- Use shallow metal pans for best results and heat transfer
- Check drain and seals

Incineration

- Treatment of choice for animal bedding, carcasses and pathological wastes; but not plastics!
- Reduces volume of waste by up to 95%
- May allow energy generated to be recovered
- Operation parameters:
  - Primary chamber: 1400°F-1800 °F (760 °C-982 °C)
  - Secondary chamber: >2000 ° F (1093 °C)
- Can generate smoke, residues with heavy metals, gases (e.g., HCl, CO, PCBs, etc.)
- May require pollution control devices, e.g., wet/dry air scrubbers, electrostatic precipitators
- Loading needs to be controlled
- May require permits

Summary

What are some of the key considerations of waste handling?

Individual Reflection

When you return to your laboratory, what are some waste handling methods you are considering changing? Adding, removing, or updating to ensure better compliance?

Laboratory Biosecurity

Learning Objectives

- Protecting biological agents and toxins in the laboratory from loss, theft, or misuse is an important aspect of laboratory operations.
- A proper biosecurity risk assessment is necessary before implementing an efficient and effective biosecurity program.
- Securing pathogens and toxins can be very different from securing other kinds of materials.
- Physical Security is only one component of a successful laboratory biosecurity program.
- Material Control and Accountability, Transport Security, and Information Security complement other security components.
- Security awareness is crucial in laboratory biosecurity.

What is Laboratory Biosecurity?

What are some unique challenges to securing biological materials in a laboratory?

What should we protect?

1) Only vials with well-characterized strains? Closely related strains? Aliquots?
2) Genetic materials? Reagents? Vectors?
3) Waste?
4) Experimental Results? Sequence Information?
5) Animals?

How would the risk change if the lab worker wore a **respirator** instead of a surgical mask, and was working in a **biosafety cabinet** that had been **certified** in the **last six months**?

OR

If there were **strict physical security** measures in place at the laboratory to keep unauthorized people out?

Biosecurity Risk Mitigation

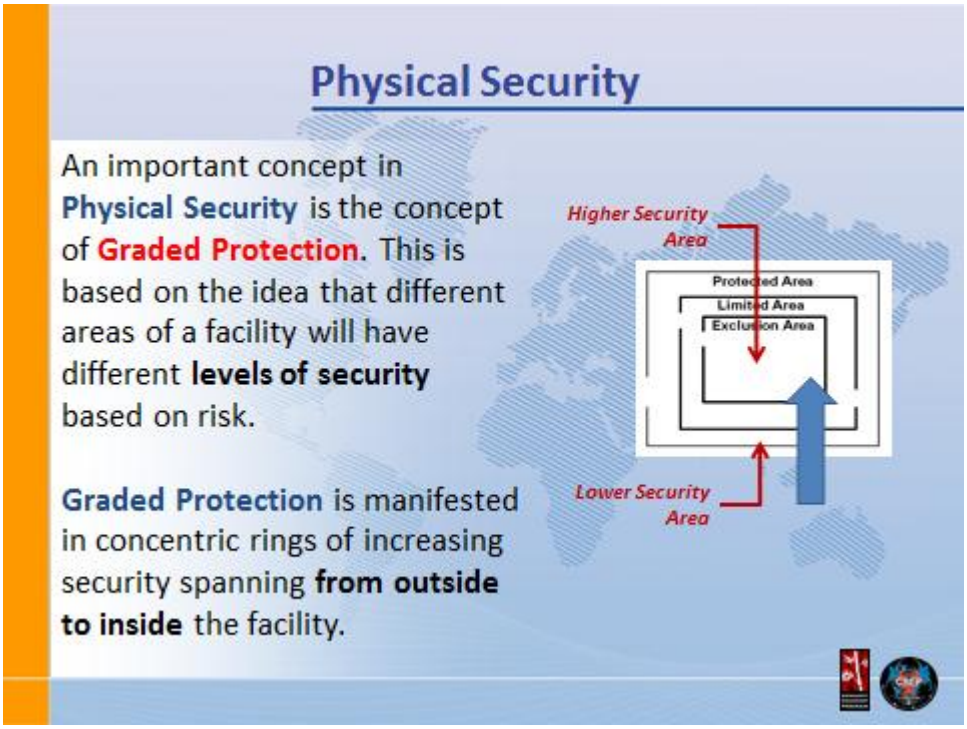
Biosecurity Risk Mitigation is the process whereby risks identified and characterized during a risk assessment are reduced through active intervention, be it physical or procedural.

Biosecurity Risk Mitigation should be based on a Risk Assessment including analyzing hypothetical scenarios with a defined agent, adversary, and a particular way that adversary will attempt to steal and/or misuse the biological material.

Five Pillars of Biosecurity Risk Mitigation

1) Physical Security - is the assurance of safety from physical intrusion
2) Personnel Management - in the context of biosecurity, is the assurance that the people that are given access to sensitive biological materials **should** have that access.
3) Material Control & Accountability - is the assurance that there is an awareness of what exists in the laboratory, where it is, and who is responsible for it.
4) Transport Security - is the assurance that the same rigorous processes that protect biological materials in the laboratory follow those materials when they are transported outside laboratory areas.
5) Information Security - is the assurance that the **sensitive** and **valuable** information stored in a laboratory is protected from theft or diversion.

Physical Security



Concentric layers of Graded Protection

Property Protection Areas (**Low risk assets**)

- Grounds
- Public access offices
- Warehouses

Limited Areas (**Moderate risk assets**)

- Laboratories
- Sensitive or administration offices
- Hallways surrounding Exclusion Areas

Exclusion Areas (**High risk assets**)

- High containment laboratories
- Computer network hubs

3 Principles of Physical Security:

- **Detection**
- **Delay**
- **Response**

We will also cover **Access Control**, which is another important, overall, aspect of physical security.

Detection

Intrusion Detection can be as complicated as a closed-circuit television system, infrared and motion sensors, and guards patrolling throughout the facility.

Or, it could be as simple as good training of laboratory staff and a procedure to call someone in case a suspicious person is noticed in the laboratory.

Delay

Delay is simply the act of slowing down an intruder's progress in your facility long enough so that the adversary may be detected, assessed and responded to.

There are many ways of delaying an intruder

- **Perimeter Fencing**
- **Solid doors with locks**
- **Bars on windows**
- **Magnetic switches on doors**

Response

Response is the act of alerting, transporting, and staging a security force to interrupt and neutralize an adversary.

Response is tied to the overall system objective

- **Deny**: To prevent an adversary from reaching the target/objective
- **Contain**: To 'catch' an adversary before they leave with the target or before they accomplish the objective

Based on your risk assessment and scenario analysis, response can range from implementing a guard force in your facility to establishing a line of communication with your local police force.

Access Control

Access Control is another important aspect of biosecurity. It is the mechanism to determine and control authorized entry into secured areas. **Access Control** also provides capability to delay or deny unauthorized personnel.

Something you have

Key

Card (Credential)

Something you know

Personal Identification Number (PIN)

Password

Something you are

Biometric feature (i.e., fingerprints)

Personnel Management

Personnel Management in the context of biosecurity, it is the assurance that the people that are given access to sensitive biological materials should have that access.

The Objectives of a Personnel Management Program are to:

Understand that human factors can significantly impact the success of biorisk management.

- To reduce the risk of theft and fraud
- To reduce the risk of scientific misconduct
- Etc..

To support the procedural and administrative access control requirements

These are some factors that can influence **Human Performance**

- **Job**
  - Setting
  - Values
- **Individual**
  - Personalities
  - Values
- **Organization**
  - Expectations
  - Assessments
- **Personnel Training – Security Awareness**
- Promoting **security awareness** in employees is one of the most important ways breaches in security can be recognized.
- Lab workers should be **aware** of who should be and should not be in their work areas.
- A person with the wrong type of badge, or simply someone you don't recognize in your part of the building, should be asked: "may I help you?" or "who are you?" and, if necessary, reported to building security.

Material Control and Accountability

Material Control & Accountability is the assurance that there is an awareness of what exists in the laboratory, where it is, and who is responsible for it.

The Objective of **MC&A** is to:

- **Ensure the complete and timely knowledge of:**
    - What materials exist
    - Where the materials are
    - Who is accountable for them
- **Objective is NOT to detect whether something is missing.** This could be impossible. The objective is to create an environment that discourages theft and misuse by establishing oversight.
- Most laboratories already control and track their samples for scientific reasons. The emphasis here is that this is also important from a security perspective.

Key Issues in **MC&A**

- What materials are subject to MC&A measures?
- What operating procedures are associated with the materials?
    - Where can they be stored and used?
    - How are they identified?
    - How is inventory maintained?
- What records need to be kept for those materials? What timeliness requirements are necessary for those records?
- What does accountability mean?
- What documentation and reporting requirements?

Material Control & Accountability

Material Control & Accountability

What information should we keep track of?

Agent	Quantity	Form	Detail	Scope
Which agents?	Any amount of a replicating organism can be significant.	Repository Stocks, Working Samples, yes...	Materials as Items	Laboratory Strains? Wild-type?
Only viable organisms? Whole org. or just DNA?	For toxins, must define a threshold amount.	What about: In host? Contamination?	Each vial as a separate inventory record?	Clinical Samples?

Slide 34

Material Control & Accountability

Material **Control** & Accountability

- **Control is either...**
  - Engineered / Physical
  - Administrative

- **Containment is part of material control**
  - Containment Lab / Freezer / Ampoule
- **Procedures are essential for material control**
  - For both normal and abnormal conditions

Slide 35

Transport Security

Transport Security is the assurance that the same rigorous processes that protect biological materials in the laboratory follow those materials when they are transported outside laboratory areas.

- Aims to reduce the risk of illicit acquisition of *high-risk* biological agents
- Relies on chain of custody principles and end-user agreements

High risk agents are routinely shipped worldwide for diagnostic and research activities

- A local, national, and international concern
- There is a need to develop a common standard, harmonize regulations for security

Chain of Custody (CoC)

Aims to protect sample by documenting…

- All individuals who have control of sample
- Secure receipt of material at appropriate location

Chain of custody documentation includes…

- Description of material being moved
- Contact information for a responsible person
- Time/date signatures of every person who assumes control

So, we want to keep our high-risk samples secure during transport. What should you do?

- Properly package all materials
- Require a responsible authority to pre-approve all transport
- Advise eligible receiving party of transport
- Document transport in lab records
- Ensure only trustworthy people handle the samples
- Physically secure samples in transit with special packaging and/or locks
- Control movements and document in delivery records
- Use timely shipping methods
- Maintain a Chain of Custody
- Request notification of receipt

LAB

Information Security

Information Security is the assurance that the sensitive and valuable information stored in a laboratory is protected from theft or diversion.

What kind of information in a laboratory should be protected? List your ideas here**:**

1.

2.

3.

4.

5.

Information Security may not the most obvious area of biosecurity, but a failure here could have very severe consequences in terms of securing pathogens and toxins.

The Objective of **Information Security** is to:

Protect information that is too sensitive for public distribution

- Label information as restricted
- Limit distribution
- Restrict methods of communication
- Implement network and desktop security

Biosecurity-related sensitive information

- Security of dangerous pathogens and toxins
  - Risk assessments
  - Security system design
- Access authorizations

Identification, Control, and Marking

Identification

- Designated sensitivity level
- A review and approval process aids in the identification of sensitivities
  - Critical prior to public release of information

Control

- Individual responsible for control of sensitive information
  - Physical security
  - Communication security
- In the US, in order to refuse public access upon request, information must be exempt from the Freedom of Information Act

Marking

- Sensitivity level designation
  - Top and bottom of each page / cover sheet
- Marking and control methods should be well understood by those working with information

Communication and Network Security

Communication Security

- Mail, email, or fax security is required
- Limited discussions in open areas
- Information should only be reproduced when needed and each copy must be controlled as the original

Network Security

- Firewalls
- User authentication
- Virus protection
- Layered network access
- Desktop security
- Remote and wireless access controls
  - Encryption
  - Authentication

Security Considerations for Network Systems

Administrators have full control

- The ultimate insider

Protect the system using procedures

- Two person control
- Configuration management
- Password control

Restrict operator privileges

Provide physical protection for equipment

Backup equipment and procedures must be provided to maintain security

Emergency power and uninterruptible power supply required for computers

In your group, identify the people that may have or need to have access to the laboratory and/or the laboratory areas. Write each type of person on a sticky note. Group the people of you identified into categories.

What are the categories of people and how did you come up with them?

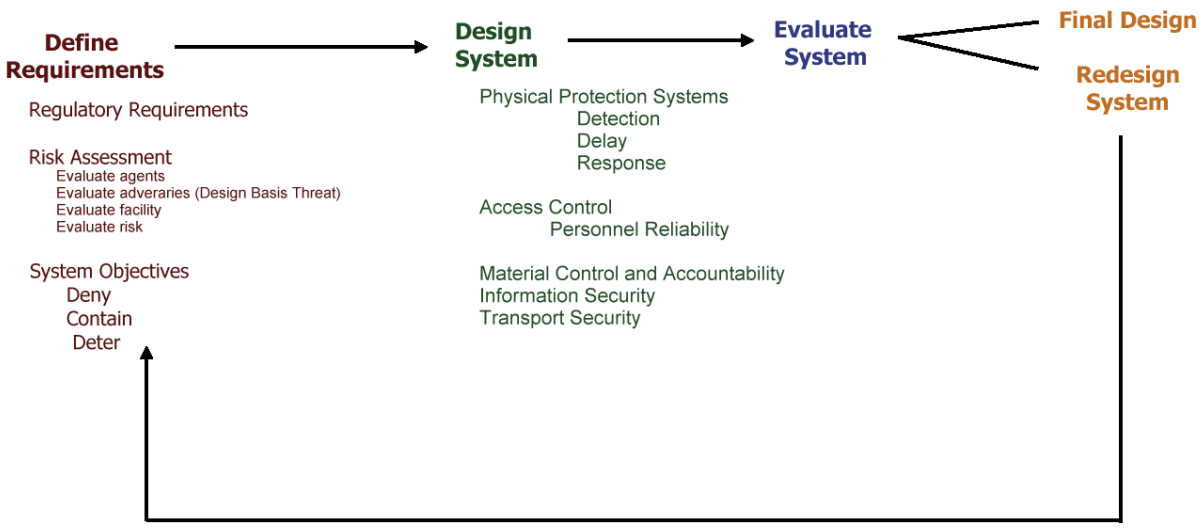
For each category, list the risks associated to and from the people on the flip chart.

Category of People	Risks associated to the people from the laboratory	Risks associated from the people to the laboratory

For each category, what measures you should consider before giving them access to the laboratory? Consider, do all groups require the same access? Do all groups require the same measures?

Category of People	Measures which can reduce the potential to or from the person	Measures which can reduce the consequences to or from the person

The following are the key principles of a biosecurity system:



The key engineering control elements are the physical security components. They fall into the following categories:

- **Detection**
  - Intrusion Detection is the process to determine that an unauthorized action has occurred or is occurring
  - Detection includes sensing the action, communicating the alarm, and assessing the alarm
- **Delay**
  - Slowing down an adversary's progress
- **Response**
  - The act of alerting, transporting, and staging a security force to interrupt and neutralize the adversary
  - Mitigation and recovery interface with the response function
- **Access Control**
  - The mechanism to 'by pass' the physical security system

How the security measures are selected should be based upon the risk assessment and system objectives.

Case Examples

1. Medical technologists are using blood cultures to diagnosis *Brucella melitensis* using commercial blood culturing systems.

2. Medical technologists are using clinical stool samples to look for diarrheal diseases with a focused look for MDR strains of *Shigella sonnei.* The technologists are culturing the bacteria and upon identification of *Shigella* strains are conducting sensitivity tests looking for MDR strains.

3. A bovine has been exhibiting signs of FMD, a veterinarian collected nasal swabs which have been sent to the laboratory for testing. Testing will be done using RT-PCR.

4. A researcher is conducting drug sensitivity studies of TB positive human samples to determine if strains are MDR or XDR. Studies are done in culture tubes.

In-Processing

In your group, outline the steps you should consider prior to allowing someone access into the laboratory based upon your example. What are some considerations for these requirements?

Out-Processing

Outline the steps you should consider in out-processing based upon your example. What are some considerations for these requirements?

Visitor Controls

Outline the steps you should consider in establishing visitor controls based upon your example. What are some considerations for these requirements?

Review

- What are the reasons for limiting laboratory access?

- How do you determine if someone can or should have access?

- How can you control access?

Incident Response

Learning Objectives

- Be able to discuss the differences between an accident and an emergency.
- Be able to explain the importance of knowing who to contact and when.
- Be able to explain the importance of addressing issues in SOPs.
- Be able to explain the importance of communication with emergency responders and others, such as the press.

What can go wrong?

In your group, list things that can go wrong in a laboratory.

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.

11.

12.

13.

14.

15.

Incidents Are Not Necessarily Emergencies

- **Incident**
  - An event that's likely to have adverse consequences
- **Emergency**
  - Unanticipated circumstances resulting in need for immediate action

Which of the items on the list you created are emergencies?

Incident Drill

In your group, develop a procedure for responding to an incident. Each group will be assigned one incident to handle. You will develop the procedure and then conduct a drill on this procedure. The drill will be conducted as a demonstration to the rest of the class. You will act it out in the provided laboratory space and you can practice it prior to demonstration. You can use PPE as needed as well as other supplies.

1. What is the incident? Is this an emergency?

2. Do you need to contact anyone regarding this incident? Are they internal to your facility or external?

3. How will you communicate with external responders? What should you communicate with the public or press? And when?

Develop a Standard Operating Procedure for your incident

Brief description of incident:

Summary of laboratory environment including PPE and any special equipment in use prior to incident:

Required PPE for incident Response:

Relevant Contact Information:

Detailed Procedure Steps:

Summary

What are some of the key considerations when thinking about incidents?

Individual Reflection

Based upon the discussions and the activities, are there additional response plans needed at your facility or plans which need to be updated?

Performance

Learning Objectives

- Be able to explain the importance of performance in the AMP model
- Understand the three components of performance
- Appreciate the CWA 15793 as an important international BRM standard

What is performance?

- Performance is the way in which someone or something functions
- Performance is the result of all the efforts of a company or organization
- Performance improves biorisk management: you know that your system works and is sustainable, and that the risk is acceptable

Performance Scenario – TB

An employee of a waste transport and disposal company was diagnosed with Tuberculosis. After his diagnosis, he recalled an incident in which waste leaked from biohazard waste bags he carried from the local "TB Reference Laboratory," but did not report it at the time. During the same period, he visited relatives and went to public places in a country where TB was prevalent. One of his relatives in that country had just started TB treatments during his visit. The laboratory did not know about his infection until it was notified of this person's pending lawsuit, which claimed that the facility had not sufficiently treated the waste. Laboratory tests on samples from his lungs did not clearly point to a laboratory-based exposure. The waste transport and disposal company does not maintain an employee health monitoring program.

Upon review of lab and autoclave records, it was identified that on one day out of five the autoclave temperature, pressure, and time recordings were not available. Some staff seemed to think that this may have been because the autoclave printer ran out of paper on that day. The reference lab does not perform regular validation of its autoclaves.

Six months prior to this incident, in an internal occupational health and safety audit, it was recognized that autoclave printout records were kept in a drawer that was also used to store laboratory supplies. The internal audit was reviewed, and the laboratory manager determined that these records should be stored in a more appropriate and secure location. Another issue identified during the audit was that employees were not aware of autoclave maintenance procedures. The assistant laboratory manager was ordered to secure the records in a specific location and train lab personnel in autoclave procedures. However, he subsequently went on extended leave of absence, and no other effort was made to correct these problems.

The lawsuit resulted in a $5 million fine for the laboratory because the laboratory could not prove that the waste was treated appropriately as stipulated in its contract with the waste transport and disposal company.

In your group, list some of the performance issues or problems in the scenario.

Issue/Concern or Problem	
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	

Cataract University Scenario

Amil works in a Biosafety Level 2 research lab at Cataract University studying anthrax vaccines. He recently visited the emergency room with a serious skin infection on his neck. Doctors determined this infection was caused by *Bacillus anthracis* and started treating him with antibiotics. He is expected to make a full recovery.

Amil was surprised to learn of this diagnosis because he only works in the lab with the Sterne strain of *Bacillus anthracis,* a non-lethal strain used to vaccinate animals. Although the high containment lab in the adjacent building works with the fully virulent strain, Amil never enters there.

Upon learning of the infection through the news, the lab director asked for a study to be done to determine what went wrong and whether or not Amil contracted the agent in the lab. Amil reported that he had been working with Barbara two weeks prior to grow up cultures of the non-lethal, live vaccine strain. Barbara was working in the Biological Safety Cabinet (BSC) to prevent contamination. After transferring a small amount of broth culture to a micro-centrifuge tube, Barbara sealed the tube and wiped it down with alcohol before transferring the tube to Amil, who placed the tubes in a labeled container and walked them down the hallway to put them in a common use refrigerator. Amil was not wearing gloves during the process as he explained "I was not directly handling the agent and Barbara was wiping them down with alcohol so I did not think there was anything to worry about." Neither researcher was aware of the fact that alcohol would have little effect on Bacillus spores.

The lab director suggested that the cultures in the lab be tested to determine whether or not the strains were indeed the vaccine version or the fully virulent strain. However, the samples turned up missing after a search of the common refrigerator where they had been stored. No one is sure what happened to them. The custodian cleaned out the refrigerator the week before and may have inadvertently tossed them in the trash but he does not remember. Fortunately, Barbara saved some of the stock solution and upon testing was surprised to find that it was the fully virulent strain of *B. anthracis* and matched the strain that was cultured from Amil's lesion. Barbara had ordered the vaccine strain from Acme Labs several months ago.

When questioned about the possibility of sending the wrong strain to Cataract University, a manager at Acme lab reported that it is very unlikely because they only shipped the virulent strains to labs that are registered with Acme and Cataract was not registered. However, the manager did concede that their shipping supervisor happened to be on vacation when the shipment was sent to Cataract so some of the records were not kept during that period.

In your group, identify problems with assessment, mitigation or performance.

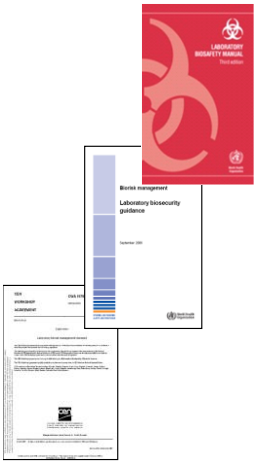
Issue/Concern or Problem	Indicate whether this is a problem with A, M, or P	CWA Section
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		

Introduction to CEN

International Laboratory Biorisk Management Documents

Technical: World Health Organization

- Laboratory Biosafety Manual (2004)
- Biorisk Management: Laboratory Biosecurity Guidance (2006)



Management: CEN Workshop Agreements

- CWA 15793 Laboratory Biorisk Management Standard
- CEN WS 55 – CWA 15793 Guidance Document (under development)
- CEN WS 53 – Biosafety Professional Competence

What is CEN?

- **CEN = Comité Européen de Normalisation**
- **CEN has 30 national members**
- **Produces technical specifications, technical reports, and European Standards (EN)**

- **CEN Workshop Agreements (CWA) produced by**
  - Any interested parties
  - Consensus documents

CWA 15793: Laboratory Biorisk Management

- **Developed by 76 participants from 24 countries**
- **Is a management system standard consistent with other international standards such as**
  - ISO 9001 / 14001 and OSHAS18001
- **The Standard is performance oriented**
  - Describes what needs to be achieved
  - How to do it is up to the organization
- **Does not replace national regulations**
  - Compliance with regulations is mandatory under CWA 15793
- **Designed to be comprehensive blueprint for biosafety & biosecurity (biorisk) program**
  - Risk-based; applicable to broad range of organizations, not just high containment labs

Purpose of the CWA 15793:2008

The Standard is used for:

- Improving overall laboratory biorisk performance
- Increasing awareness and the adoption of performance approaches for biosafety and biosecurity
- Improving international laboratory collaboration and safety harmonization
- Support laboratory certification/accreditation, audits/inspections

CWA 15793:2008

International Approach

- Extensive definition section
- Not country specific
- Based on international, acceptable best practices
- Local solutions possible
- The Standard is based around the current WHO Biosafety and Biosecurity Guidelines

Example: Waste Management

4.4.4.5.3 Waste Management

The organization shall establish and maintain an appropriate waste management policy for biological agents and toxins.

- **The standard is not a technical document**
- **Describes what needs to be achieved, but allows organizations to determine how best to achieve those objectives**
- **Provides Biorisk management framework for the day-to-day functions of the institute / organization**
  - **During normal operations and times of emergency**

Reasons for Implementing CWA 15793

Enables organizations to:

- Establish and maintain a biorisk management system to control or minimize risk to acceptable levels to employees, the community and others
- Provide assurance that the requirements are in place and implemented effectively
- Provide a framework that can be used as basis for training and awareness raising
- Seek and achieve certification or verification by an independent third party

Document available on CEN website

ftp://ftp.cenorm.be/PUBLIC/CWAs/workshop31/CWA15793.pdf

The full standard is also included on your CD

CWA 15793:2008

Examples of topics covered:

- Biorisk Management Policy
- Hazard identification, risk assessment and risk control
- Roles, responsibilities and authorities
- Training, awareness and competence
- Operational control
- Emergency response and contingency plans
- Inventory monitoring and control
- Accident and incident investigation
- Inspection and audit
- Biorisk management review

Individual Reflection

How does AMP apply to your own lab?

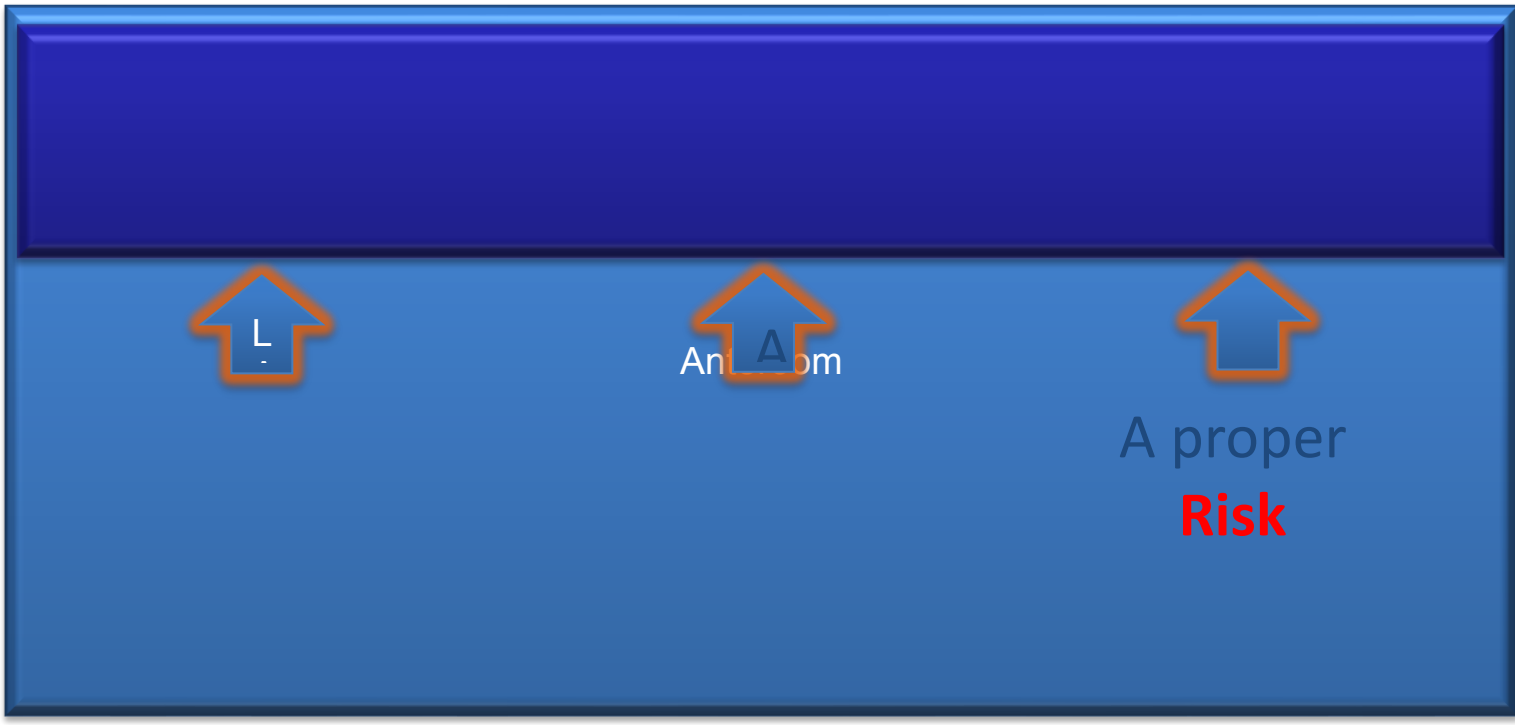
How could you improve biorisk management at your own lab, short-term and long-term?

What would be the challenges of implementing AMP?

What would be the benefits of implementing AMP?

Summary

The AMP model



- Assessment = Plan, Do, Check, Act
- Mitigation = Plan, Do, Check, Act
- Performance = Plan, Do, Check, Act

Laboratory Activities (Day 2)

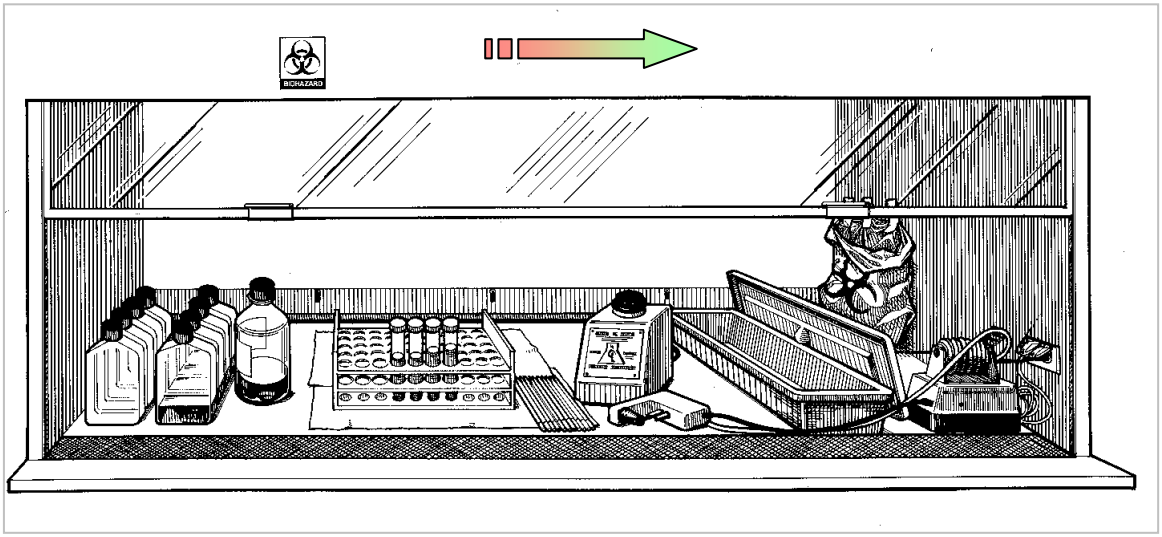
Organized Workspace Exercise

Learning Objectives: Upon completing the exercise, the student should…

- Be able to set up a biosafety cabinet properly prior to work.
- Be able to work in a biosafety cabinet using the clean to dirty principle.
- Be able to properly clean up a biosafety cabinet after use.

Clean to Dirty

The principle of "Clean to Dirty" helps limit contamination and accidents by organizing your workspace. When properly organized, work flows from one end of the workspace to the other, from "clean" areas where materials have not yet been used, to "dirty" areas where contaminated materials then need to be disposed. Waste can be collected near the end of the workflow and kept isolated from clean materials until it can be properly disposed of.



Look inside your biosafety cabinet.

Is the workspace properly organized for laboratory work?

Activity

Set the items up within your biosafety cabinet to conduct a series of 1:10 serial dilutions of the red stock solution, following the "Clean to Dirty" principle. **Write** down the items you need below:

You can remove any other items you do not need for the dilutions.

Questions

Can the arrangement of items appear different from the image in the previous page? Explain.

How might the arrangement you chose help lower laboratory safety risks?

What are the safety risks posed by an overfilled biosafety cabinet (i.e. too many items inside)?

Streaking Plates Exercise

Learning Objective: Upon completing the exercise, the student should…

- Be able to identify proper techniques in streaking, including mitigation of fire hazards.

Brief Description of the Exercise

In this exercise, you will streak one agar plate with *Escherichia coli* strain K-12 bacteria.

Materials

What materials and equipment will you need?

Risks

What are the risks of this experiment?	How do you mitigate these risks?

Procedure

1. **Label** the agar plate.

2. **Remove the** cap from the culture tube and if possible **keep** it in your hand. **Insert** a sterile disposable loop into the broth and **remove** it. **Replace** the cap on the culture tube.

3. Streak the plate using the method described below. **Do this** while **minimizing** contamination of the plate (**don't leave** the plate surface unnecessarily uncovered, one methods is to **lift** the lid but **keep it covering** the plate while streaking).

4. **Turn** your plate over and **draw** a "T" on the bottom. **Turn** the plate back over so that the lid is on the top side.

5. **Using** the sterile loop, **obtain a** sample of bacteria and **streak** it in the topmost section of the plate. **Be careful** not to go outside the lines.

6. **Move** back into the first section once or twice to transfer some bacteria to the loop, then without going back in to the first section again **complete** streaking in the second section.

7. **Move back** into the second section once or twice to transfer some bacteria to the loop, then without going back into the second section again **complete** streaking the third section.

8. **Place** the plate upside down (lid down) in the incubator.

Questions

Would you change anything to this procedure to reduce laboratory risks?

Questions (continued…)

How would the risks have changed if you had been streaking plates with pathogenic *Escherichia coli* O157:H7?

How would the risks have changed if you had been using a metal loop and a Bunsen burner to sterilize instead of pre-sterilized plastic loops?

How would the risks have changed if your partner had been eating a sandwich and drinking a cola while you streaked the plates?

Serial Dilutions Exercise

Learning Objective: Upon completing the exercise, the student should…

- Be able to explain the advantages of using proper pipetting techniques and identify the different pipette aids available.

Brief Description of the Exercise

In this exercise, you will conduct a series of 1:10 dilutions of oil-based fluorescin in vegetable oil.

Materials

What materials and equipment will you need? What type of pipette aid is best? What kind of tubes?

Procedure

Using the materials you have chosen, develop a procedure to conduct these dilutions.

Risks

What are the risks of this procedure?	How do you mitigate these risks?

Questions

How would the risks have changed if you had been conducting dilutions of a culture of multi-drug resistant *Mycobacterium tuberculosis*?

How would the risks have changed if you had been vortexing tubes for 15 seconds between dilutions?

Summarize the potential risks of pipetting liquids in a bench top.

Egg Inoculation Exercise

Learning Objective: Upon completing the exercise, the student should…

- Be able to discuss the safe handling and disposal of sharps.
- Be able to discuss the risks of handling fragile biological specimens.

Brief Description of the Exercise

In this exercise, consider the inoculation of an egg with a small volume of liquid, as well as the extraction of a small volume of sample liquid from a previously "inoculated" and incubated egg. PLEASE BE CAREFUL WITH THE SHARPS IN FRONT OF YOU. You will NOT be conducting an actual inoculation.

Materials

1 clean egg
1 inoculated egg
Phosphate Buffered Saline (PBS)
Bee's Wax
1 Syringe
1 Needle
A flashlight
Forceps
1.5 mL tubes
Markers
"Exacto" Knife

Any other materials you might need?

Procedure

Develop and **write down** a procedure for inoculating one egg and harvesting from a second. What would you need to do to make a clean puncture in an egg using the materials in front of you?

If you have any questions, please ask an instructor.

Risks

What are the risks of these procedures?	How do you mitigate these risks?

Questions

How would the risks have changed if you were using a biosafety cabinet for this experiment?

Questions (continued…)

How would the risks have changed if you had been inoculating eggs with a sample suspected of containing highly pathogenic avian influenza virus?

How would the risks have changed if you were using a smaller gauge (thicker) needle?

How would the risks have changed if you were inoculating and drawing from a mouse instead of an egg?

How would the risks have changed if you were harvesting 100 eggs in a row?

Day 2 Laboratory Activities Reflection Exercise

Are there any lessons from today's laboratory activities that will influence how you approach safety policies in your workplace when you return?

Laboratory Activities (Day 3)

Biosafety Cabinet Airflow Exercise

Learning Objective: Upon completing the exercise, the student should…

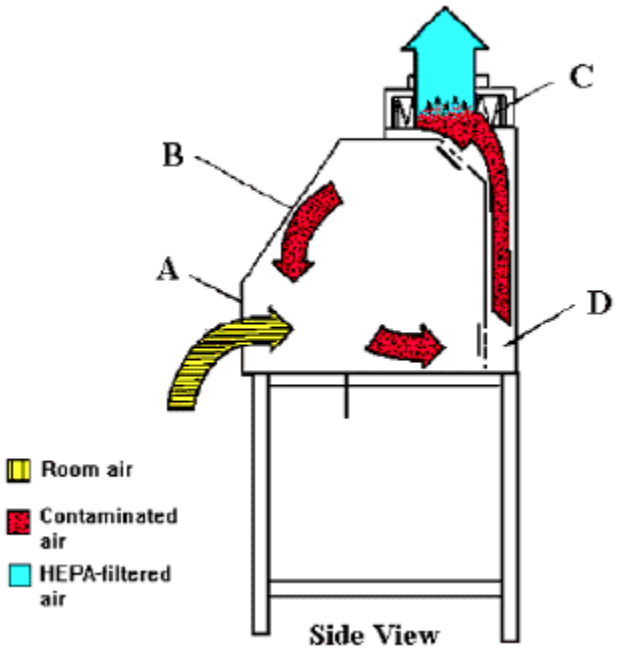
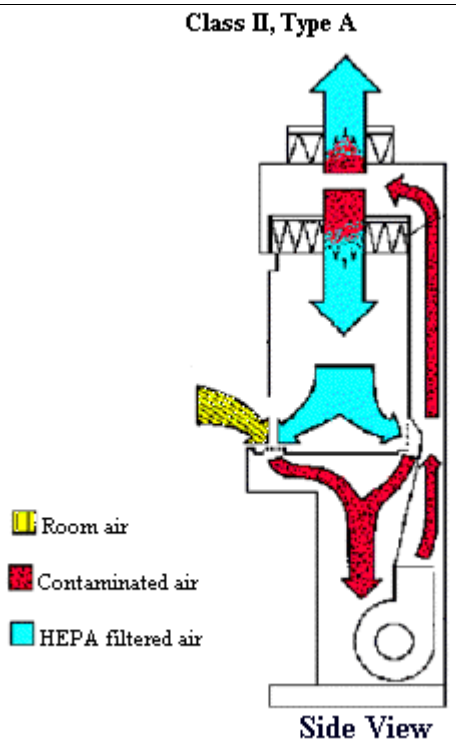
- Be able to explain the differences between Type A biosafety cabinets, Type B biosafety cabinets, and chemical hoods.
- Be able to describe the flow of clean and dirty air within a biosafety cabinet.

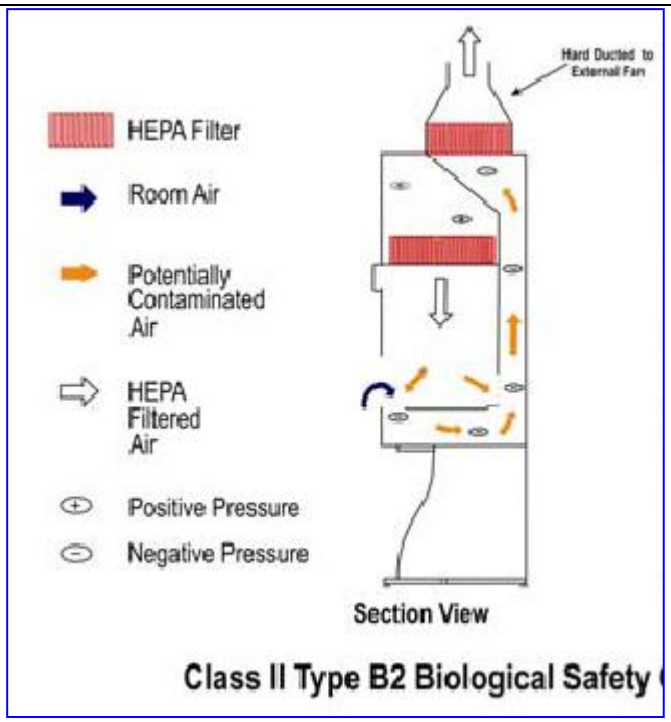
Background Information

Biological safety cabinets, or BSC, are a primary means of biological containment. There are three design types: Class I, Class II, and Class III. All work by controlling how air flows into and out of the cabinet, and by using HEPA (high efficiency particulate absorption) filters, which filter 99.97% of particles 0.3 microns in size, including viruses, bacteria, and prions.

	Protects Personnel	Protects Product	Protects Environment
Class I BSC	X		X
Class II BSC	X	X	X
Class III BSC	X	X	X
Chem. Fume Hood	X		
Lam. Flow Clean Bench		X	
Isolator	X	X	X

Questions

Based on the table in the previous page, do you think this a Class I or Class III BSC**?**	
	Based on the airflow diagram, how would this Class II Type A protect personnel? Protect product? Protect the environment?

Questions (continued…) What appears to be the functional difference between the Class II Type A cabinet above and the Class II Type B cabinet to the right?	

Use the smoke stick to visualize the airflow in the biosafety cabinet in front of you and **record** your observations below.
• Look at the inward airflow at the front of the sash. What happens if some of the holes in the front grill are covered up with the absorbent mat?

Questions (continued…)

- While looking at the inward airflow at the front of the sash, have your partner walk quickly by the biosafety cabinet. What happens?

- Pretend to work in the biosafety cabinet while your partner holds the smoke stick near the front sash. What happens if you need to reach outside the cabinet for supplies?

- Look at the air split in the center of the cabinet. Where is the best place in the cabinet to conduct a procedure that generates aerosols?

Laboratory Airflow Exercise

Learning Objective: Upon completing the exercise, the student should…

- Be able to explain how HEPA filters work.
- Be able to explain the principle of air moving from clean to dirty.
- Be able to explain the mechanisms for achieving and verifying laboratory airflow.

Background Information

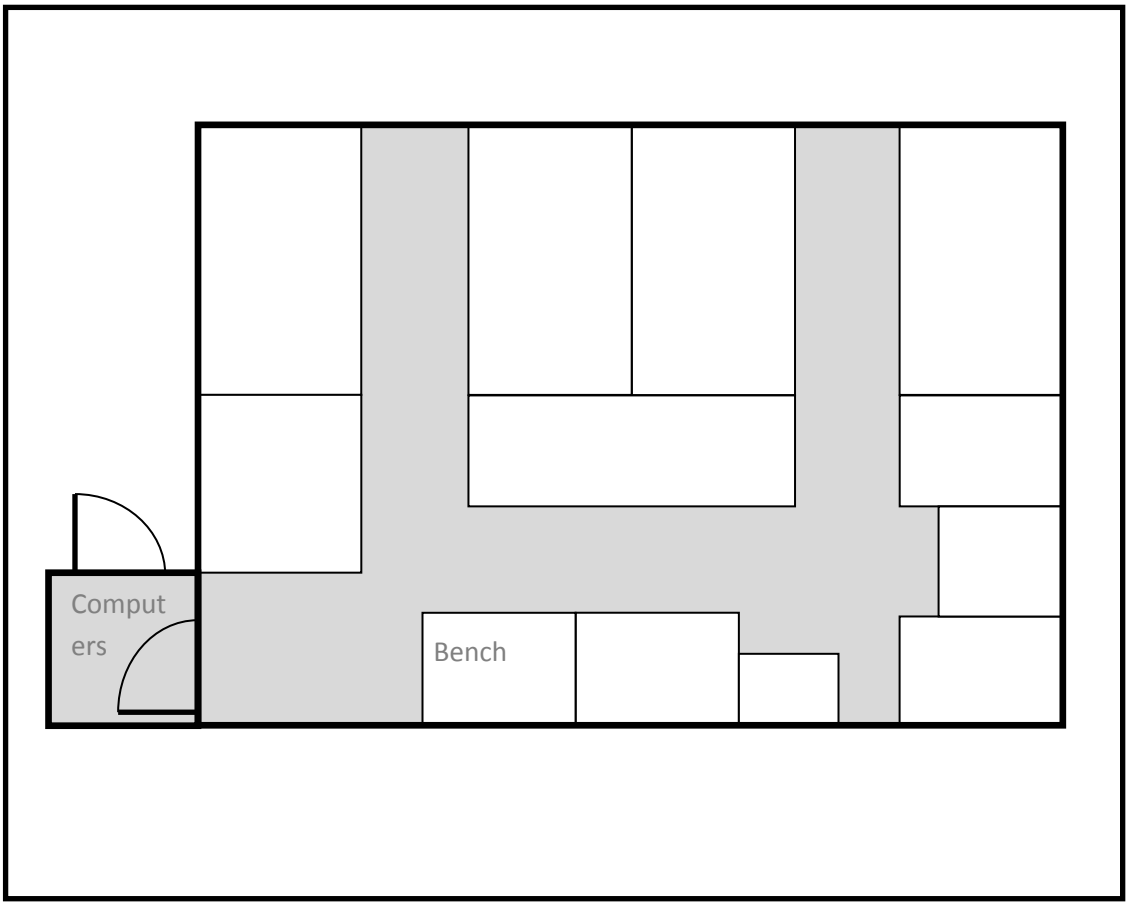
In a laboratory in which infectious aerosols are created, it may be desirable to control the flow of air into and out of the room in order to prevent contamination of the outside world. Air should flow from "clean to dirty", and dirty air should be either filtered or exhausted in such a way to prevent unintentional exposures to potentially dangerous biological materials.

Activity

Please **draw** arrows to show the direction air <u>should</u> flow in the laboratory below.

Background Information

Biological safety cabinets, or BSC, are a primary means of biological containment. There are three design types: Class I, Class II, and Class III. All work by controlling how air flows into and out of the cabinet, and by using HEPA (high efficiency particulate absorption) filters, which filter 99.97% of particles 0.3 microns in size, including viruses, bacteria, and prions.



Air flow can be controlled by creating negative and positive pressure areas in a space using mechanical air handling systems. If fans extract more air from one room than they do from another, we say the first has negative air pressure (in relation to the second room). If air can pass between these two rooms, air will then flow from the room from which relatively less air is extracted to the room in which relatively more air is extracted; that is, from an area of higher air pressure to an area of lower air pressure.

Questions

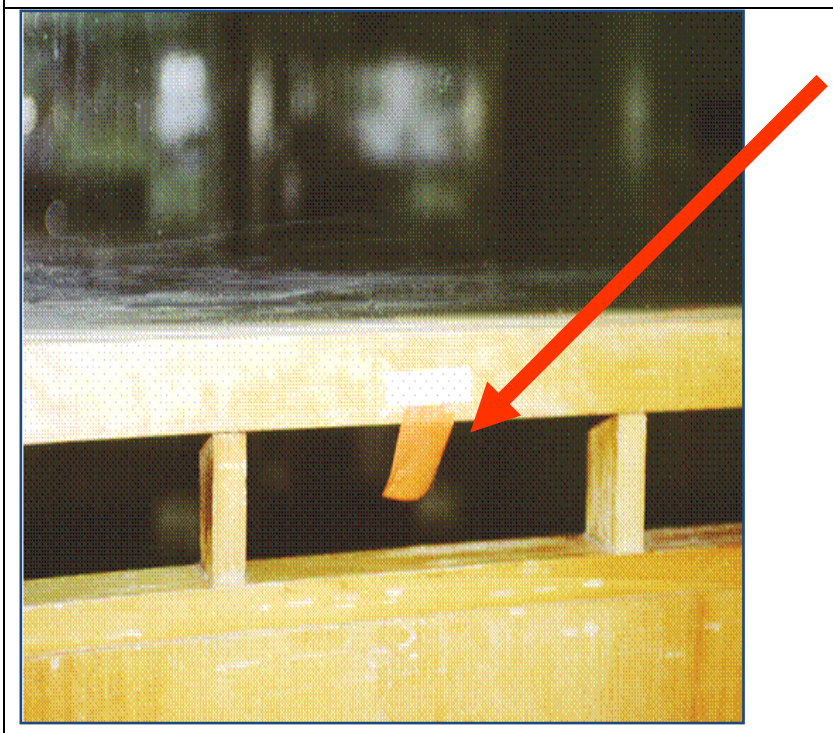
Therefore, following the "clean to dirty" principle, do you think the mechanical air handling systems in this laboratory should extract relatively more air from the laboratory, or less air, when compared to the anteroom? Explain.

Activity

Verify with your partner that the air in our laboratory is flowing in the directions you noted above. Use the items on the anteroom table to make this determination. **Describe** below what you determined was the direction of the airflow and how you exactly you determined it.

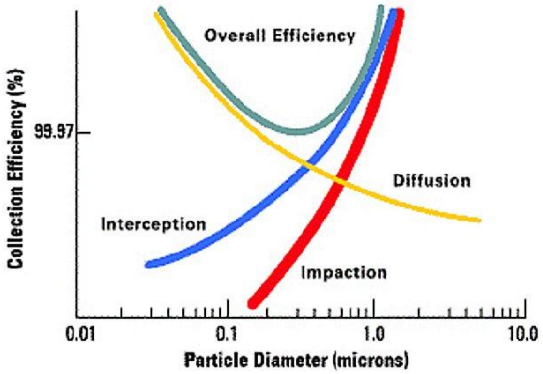
Questions (continued…)

Complete the following :

		Is this situation safe? Explain.
	This image was taken outside the laboratory.	
	This image was taken outside the laboratory.	
	The gauges show the air pressure in the Laboratory is less than the air pressure in the Anteroom, and the air pressure in the Anteroom is less than the air pressure in the Outside World.	

HEPA Filters

The following is a HEPA (high-efficiency particulate absorbing) filter. These filters remove, by design, 99.97% of airborne particles 0.3 microns in size, and even higher percentages of particles larger as well as *smaller* than 0.3 microns.



Questions (continued…)

If you were concerned about infectious aerosols and contaminating the outside world with a biological agent, where would you want to find HEPA filters in this laboratory?

Reflection

How might you assess the importance of controlling the flow of air in a particular laboratory?

Equipment Hazards Exercise

Learning Objective: Upon completing the exercise, the student should…

- Be able to explain routes of exposure and identify potential hazards with different laboratory equipment.

Background Information

Exposure to a biological agent can occur by one of four routes:

- Percutaneous, or by a puncture through the skin
- Contact, through mucous membranes
- Inhalation, or through the lungs
- Ingestion, or through the gastrointestinal system

Different equipment in the laboratory can facilitate exposure through one or more of these routes if it is used improperly, is damaged, or malfunctions. Some equipment can cause harm that does not result in an exposure to a biological agent, but which can be significant nonetheless. It is important to recognize the dangers posed by the equipment around us every day in the laboratory.

Questions

What are some ways to reduce the likelihood of:

Improper use of equipment in the laboratory?

Damage to equipment?

Malfunctioning equipment?

Activity

Please **indicate** whether the pieces of laboratory equipment below represent a hazard in any of the five listed categories. Add at least five other pieces of equipment you find in the lab and rate them as well.

	Percutaneous	**Contact**	**Inhalation**	**Ingestion**	**Other (Non-Biological)**
Bench-top Centrifuge					
5-ml Glass Test Tube					
Vortex					
Bunsen Burner					
Needles					
Glass Beakers					
Plastic Beakers					
Pipettes					
Incubator					
Autoclave					

Material Control and Accountability Exercise

Learning Objective: Upon completing the exercise, the student should…

- Be able to explain the importance of knowing what you have, where you have it, and who's responsible for it.

Background Information

Material Control and Accountability (MC&A) is concerned with ensuring that there is complete and timely knowledge of:

1. The materials that exist in the lab.
2. The locations of these materials in the lab.
3. The person accountable for these materials in the lab.

Questions

In your mind, why is the above important?

How would you design a Material Control and Accountability program for this laboratory? What materials would you keep track of? How would you keep track of them? How would you assign responsibility for them?

Questions (continued…)

Design a simple inventory sheet in the blank table below and **conduct** an inventory of the biological materials (and other materials, if you so choose) in this laboratory. **Choose** the unit of measure for the items and what fields you think are important to keep track of.

Item			**Responsible Person**	

Questions (continued…)

Discuss why you chose the units of measure and the fields that you did.

What problems did you encounter while conducting an inventory of biological agents in this laboratory?

What are some of the limitations of your sheet? Would you do anything differently in your labs?

How often should you re-inventory? Why?

Day 3 Laboratory Activities Reflection Exercise

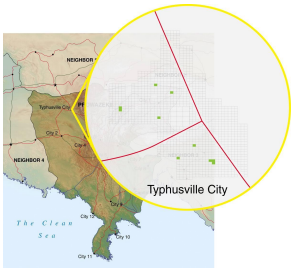
Are there any lessons from today's laboratory activities that will influence how you approach safety and security policies in your workplace when you return?

Case Studies

Introduction to the Hypothetical Facility

Typhusville City, Republic of Prowazekii

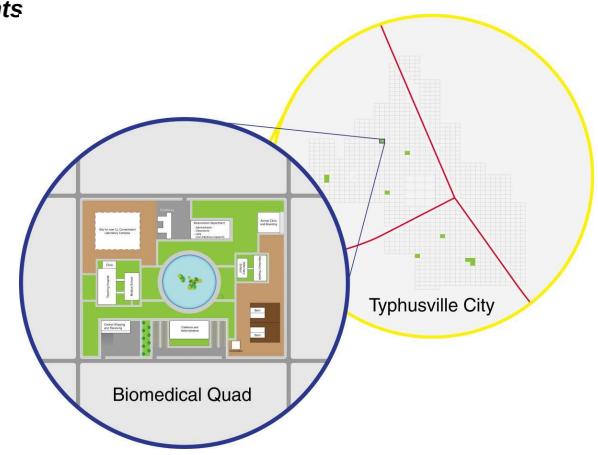
- *Government*
- *Population*
- *Economy*
- *Infectious Diseases*
- *Biotechnology/Bioscience Regulations*
- *Crime*
- *Emergency Services*



International BIOLOGICAL THREAT REDUCTION

Rickettsia National University

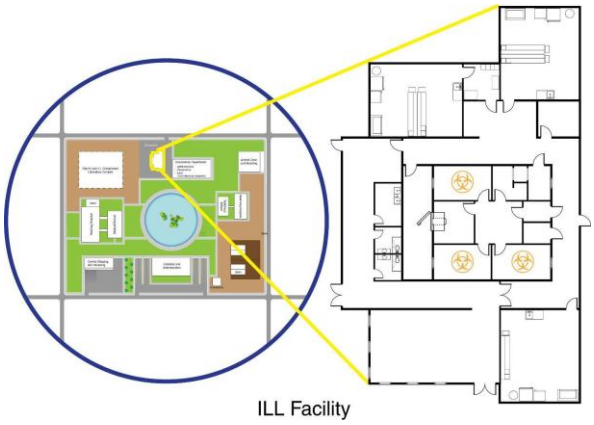
- *General Information*
- *Colleges and Departments*
- *Campus Police*
- *Memorandums of Understandings (MOUs)*
- *Security*



International BIOLOGICAL THREAT REDUCTION

Infectious Lifesciences Laboratory (ILL)

- ***Facilities***
- ***Staff***
- ***Current Research***



ILL Facility

International BIOLOGICAL THREAT REDUCTION

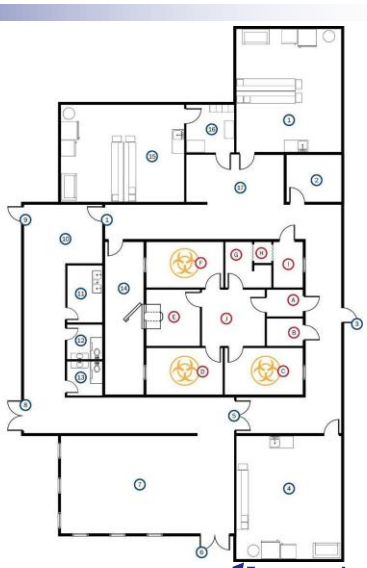
Infectious Lifesciences Laboratory (ILL)

General Facility Callouts

1. Lab 2 (BSL II)
2. Freezer
3. East emergency exit
4. Lab 3 (BSL II)
5. Limited Area entrance
6. Main building entrance
7. Offices and work areas
8. Delivery entrance / West emergency exit 1
9. West emergency exit 2
10. Break area
11. Utility closet
12. Men's restroom
13. Women's restroom
14. Autoclave access area
15. Lab 4 (BSL II)
16. Dressing room
17. Anteroom

Lab 1 (BSL III) Callouts

A. Airlock
B. Mechanical room
C. Lab 1a
D. Lab 1b
E. Autoclave
F. Lab 1c
G. Change room
H. Shower
I. Entry
J. Central area



International BIOLOGICAL THREAT REDUCTION

Infectious Lifesciences Laboratory

Hypothetical Bioscience Facility for Case Studies[1]

International Biological Threat Reduction Department

Sandia National Laboratories

Albuquerque, NM USA

www.biosecurity.sandia.gov

[1] SAND No. 2007-6186 C

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

Acronyms

ABSL1	Animal Biosafety Level 1
ABSL2	Animal Biosafety Level 2
ABSL3	Animal Biosafety Level 3
ABSL4	Animal Biosafety Level 4
AG	Australia Group
BSC	Biosafety Cabinet
BSE	Bovine Spongiform Encephalopathy
BSL1	Biosafety Level 1
BSL2	Biosafety Level 2
BSL3	Biosafety Level 3
BSL4	Biosafety Level 4
BO	Biorisk officer
BWC	Biological and Toxins Weapons Convention
CDC	US Centers for Disease Control and Prevention
EMUR	Extreme Malicious Use Risk Group
FAO	Food and Animal Organization
HMUR	High Malicious Use Risk Group
HPAI	Highly Pathogenic Avian Influenza
IBC	Institutional Biorisk Committee
ILL	Infectious Lifesciences Laboratory
LBM	Laboratory Biosafety Manual
LMUR	Low Malicious Use Risk Group
MDR	Multi-drug resistant
MMUR	Moderate Malicious Use Risk Group
OIE	Office International des Epizooties
RNU	Rickettsia National University

TB	Tuberculosis
UNSCR 1540	United Nations Security Council Resolution 1540
USDA	US Department of Agriculture
WHO	World Health Organization

Republic of Prowazekii

Government

The modern Republic of Prowazekii was established in 1752 and is divided into five provinces. The first half of the 20th century saw many changes in regime and leadership from multiple parties, but the government has settled into a stable two-party democratic government. The Executive Branch consists of the President, who serves as Chief of State and Head of Government, and the Cabinet, to which the President makes appointments. The legislative branch consists of a bicameral National Congress containing the Senate (20 seats elected by popular vote; members serve eight-year terms; half are elected every four years) and the Chamber of Deputies (60 seats; members are elected by popular vote to serve four-year terms). The Supreme Court heads the judicial branch. The president appoints judges to the Supreme Court from a list of candidates provided by the court itself. The Senate must ratify the appointments.

Population

As of the latest census, the population of Republic of Prowazekii is 52,821,286 with a density of 87 persons per km^2. The society is heterogeneous with 14 different ethnic groups. A recent survey identified 42% of the population as Christian, 35% as Muslim, 10% Buddhist, 3% Jewish, and 10% as "other". Many languages are spoken throughout the country, but English is the official working language. The Mallie Misanthropes group in the southern part of the country is seeking to secede from the Republic. This is a vibrant political movement with strong support in the southern province. A small, more radical subgroup of Mallie Misanthropes uses periodic acts of "terrorism" to draw political attention to its struggle for independence.

Economy

The economy depends primarily on agriculture, petroleum exports from off-shore oil fields, and tourism. The agricultural sector accounts for 20% of both GDP and total exports. The sector also employs 23% of the workforce.

Infectious Diseases

In general, the incidence of infectious diseases in the Republic of Prowazekii has decreased over the past two decades. Last year the government spent approximately 6% of its total expenditures on public health. Influenza is the most frequent disease of public health concern followed by food-borne illnesses. The large majority of these food-borne infections are attributed to *Salmonella sp*. Other human infectious diseases of importance include viral hepatitis B and C, which had incidences of 4.1 and 5.6 per 100,000. The country is also experiencing a reemergence of tuberculosis. There are a high number of highly infectious, potentially zoonotic diseases endemic throughout the country, including *Bacillus anthracis*, *Yersinia pestis*, *Brucella* species and Rabies Virus. Outbreaks in the agricultural industry are common. This has the potential to become a significant public health concern because of the large population who works in the agriculture industry and has close intimate contact with livestock. The Republic of Prowazekii is officially free of Foot-and-Mouth Disease and Rinderpest, but has almost annual outbreaks of avian influenza and Newcastle Disease. The country experiences occasional outbreaks of Vesticular Stomatitis.

Biotechnology/Bioscience Regulations

Several years ago, the Republic of Prowazekii's government identified bioscience as an area of potential high growth for the country, and is especially interested in promoting agricultural and health applications of biotechnology. There are few national regulations in this field. Agricultural Ministerial decree No. 1745 established procedures for

commercializing genetically modified crops. The Republic of Prowazekii lacks import and export regulations of any type, but the country would like to become a member of the Australia Group. A government committee is currently considering legislation and guidance. Republic of Prowazekii is party to the Biological and Toxins Weapons Convention (BWC). A 1982 law implemented the BWC agreements, prohibiting and criminalizing possession or use of biological weapons.

There are no laboratory biosafety regulations. Laboratories follow the World Health Organization's *Laboratory Biosafety Manual* (LBM). In response to growing concerns domestically and internationally, the country passed biosecurity regulations last year and the regulations take effect six months from now. The Biosecurity Act classifies biological agents into four categories, each with separate protocols and penalties. Group 1 agents are the lowest risk and Group 4 are the highest. Group 4 contains the agents of extreme risk, such as the Ebola and Marburg Viruses. Possession, import, export, and domestic transfer of Group 4 agents are categorically prohibited, except when specially designated by the Minister of Health or the Minister of Agriculture. Group 3 contains high risk agents, including botulinum toxin and *Bacillus anthracis*. Prior permission from the Ministry of Health or Ministry of Agriculture must be obtained in order to possess or transfer Group 3 agents. Group 2 agents are not considered high risk, but still have a significant potential for malicious use. This group includes the pathogens that cause Q fever and Tick-borne Encephalitis. Activities involving Group 2 agents do not require prior permission, but identification or possession must be reported to the Ministry of Health or Ministry of Agriculture within seven days. Additionally, records must be kept of all activities involving Group 4, 3, or 2 agents. Group 1 includes agents that are not considered a sufficient risk to require reporting of possession and transfer, but do merit some caution. Any accidents with Group 1 agents must be reported. Agents that are not specifically listed in the regulations are not currently controlled. Importantly, the law also requires that agents in Groups 1-4 be handled with appropriate biosafety controls. Facilities with any Group 1-4 biological agents must implement a risk-based approach to both laboratory biosafety and biosecurity. The government is permitted to inspect facilities to ensure that appropriate controls and record keeping are in place.

Group 4

Crimean-Congo Hemorrhagic Fever Virus

Ebola Virus

Foot and Mouth Disease Virus

Lassa Virus

Marburg Virus

South American Hemorrhagic Fever Viruses

Variola Major Virus

Group 3

Bacillus anthracis

Bovine Spongiform Encephalopathy (BSE)

Clostridium botulinum (botulinum toxin)

Francisella tularensis

Highly Pathogenic Avian Influenza (H5N1)

Severe Acute Respiratory Syndrome (SARS) Corona Virus

Yersinia pestis

Multi-drug resistant *Salmonella enterica* (serotype typhi)

Group 2

B Virus

Brucella spp.

Burkholderia mallei

Burkholderia pseudomallei

Coccidioides posadasii

Coxiella burnetti

Eastern Equine Encephalitis Virus

Exotic New Castle Disease Virus

Hantaan or other Hanta viruses

Hendra Virus

Nipah Virus

Monkeypox Virus

Multi-drug resistant *Mycobacterium tuberculosis*

Tick-borne Encephalitis Viruses

Rabies Virus

Rickettsia rickettsii

Rift Valley Fever Virus

Venezuelan Equine Encephalitis Virus

Vesticular Stomatitis Virus

Western Equine Encephalitis Virus

Group 1

Chlamydia psittaci

Cryptosporidium parvum

Mycobacterium tuberculosis

Salmonella enterica (serotype typhi)

Shigella dysenteriae (serotype 1)

Typhusville City, Republic of Prowazekii

The city covers 485 square kilometer with a population of 570,000. The median age is 34 years. Typhusville's largest employers are the public school system, Rickettsia National University, the City of Typhusville, and Typhusville General Hospital.

Last Year's Crime statistics

Crime	per/100,000 people
Aggravated Assault	4.3
Arson	0.2
Bribery	0.11
Burglary	9.25
Vandalism	15.8
Disorderly Conduct	4.2
Embezzlement	1.65
Fraud	6.93
Homicide	0.06
Kidnapping	0.62
Motor Vehicle Theft	8.42
Narcotics Offenses	5.75
Simple Assault	10.5
Weapons Law Violations	0.78
All Other Offenses	43.7

Emergency Services distance to Rickettsia National University

Typhusville City Police Department: 5 km from RNU, which is a 20-minute drive time with sirens during peak traffic and 10 minutes drive time with no traffic.

City Fire and Ambulance: 2 km from RNU, which is a 5-minute drive time with sirens even during peak traffic times.

Rickettsia National University

Overview

Rickettsia National University (RNU), located in Typhusville City, is one of the leading universities in the Republic of Prowazekii. RNU employs 4,500 people and has 18,500 students from across the Republic of Prowazekii and 25 countries. The university's highly regarded liberal arts, sciences, and engineering programs draw outstanding students from around the region. There are seven graduate and professional schools, including: College of Veterinary Medicine, College of Medicine, College of Engineering, College of Arts and Sciences, College of International Relations, the Teacher's College, and the Business College.

College of Veterinary Medicine

The College of Veterinary Medicine employs approximately 75 faculty and 195 staff. There are 220 students in the four-year, post-baccalaureate doctor of veterinary medicine program, a four-year veterinary medical curriculum of classroom and laboratory instruction and clinical rotations in the University Hospital for Animals. The College also has a graduate program (MS and PhD). Graduate fields of study within the College include comparative biomedical sciences, microbiology, immunology, pharmacology, physiology, biochemistry and cellular and molecular biology, and zoology. All fields except zoology are administered through the Biological and Biomedical Sciences Program in the College of Arts and Sciences. A core group of veterinary faculty has a newly expanded emphasis on bacterial pathogenesis and post-genomics research against microbes.

College of Medicine

There are approximately 525 medical students, 500 interns and residents, and approximately 200 graduate students and post-doc fellows studying medicine at RNU. The College of Medicine has 25 academic departments, 19 clinical and 6 basic sciences, as well as the Unit for Laboratory Animal Medicine and Department of Medical Education. Teaching, research, and clinical care often cross traditional departmental boundaries; researchers and clinicians frequently collaborate. Research specialties in the College include geriatrics and aging (focused on how genes and hormones affect the rate of aging), cancer, and neuroscience studies. In addition to their work in research and education, faculty in clinical departments provide inpatient and outpatient care at the University Hospital and Out Patient Clinic.

Biosciences Department

The Biosciences Department resides in the College of Arts and Sciences. With 41 tenured and research faculty, 66 professional staff, 75 graduate students, and 125 undergraduate students, the Department is a dynamic research and teaching environment. Facilities for cell biology, genomics, immunology, environmental microbiology, and molecular biology include the capacity for small animal studies. Large animal research is done in partnership with the College of Veterinary Medicine. The cornerstone of RNU's biosciences research program is the Infectious Lifesciences Laboratory (ILL). ILL is the country's premier bioscience research and educational institution. The laboratory primarily focuses on zoonotic emerging infectious diseases, since these are the source of almost all emerging diseases throughout the world. ILL's mission is to conduct research and development of new vaccines, drugs, and diagnostics for these emerging diseases. The Republic of Prowazekii has recently awarded RNU a grant to build a new infectious disease laboratory.

RNU Campus

Campus Police

The campus police force has a total of 50 officers. The campus police work shifts to provide coverage 24 hours per day, 7 days a week, and 365 days a year. Nights and weekend shifts consist of one deputy commander and four officers. The commander and eight officers staff the day shift. Campus police officers are equipped with the following:

- A straight baton,
- One set of handcuffs,
- A small flashlight,
- A handheld radio, and
- Keys to all buildings, doors, and gates.

The commander or deputy commander is stationed in the control station. It is approximately a 5-minute drive, 7-minute bike ride, or 20-minute walk from the campus police control station to the Biosciences Quad. The on-duty officers patrol the campus in pairs and respond to radio calls from the control station.

Threats of Concern to Campus Police

Since the establishment of the Band of Mercy in 1973, radical extremist groups sympathetic to the animal rights movement have been active in the Republic of Prowazekii, and occasionally in Typhusville. The group appears to have two goals: 1) the liberation of captive animals and 2) the destruction of capital necessary to conduct such research. However, the Band of Mercy's website instructs its followers to "take all necessary precautions against harming any animal, human and non-human."

In the past few years, anti-Genetically Modified Organism activists have become active in Typhusville. Although they have not damaged property yet, similar groups in neighboring countries have targeted facilities that conduct research on genetically modified organisms (GMOs), ***particularly*** newly constructed biotechnology laboratories.

Last year, one graduate student in chemistry was arrested on charges of manufacturing methamphetamine in a RNU laboratory.

Memorandums of Understandings (MOUs)

The University has established Memorandums of Understandings between RNU and the city police force, the fire department, and emergency medical services. All responders have campus keys and will enter standard campus buildings as needed to respond to calls. None of the responders will enter bioscience laboratories without being accompanied by RNU's biorisk officer.

Access to Campus Buildings

The University has an open campus; there is no controlled perimeter. All classroom and research buildings are unlocked from 6 am – 8 pm Monday through Friday. Buildings are locked outside of these hours.
For a given laboratory building, one master key opens the exterior doors and a second master key opens all laboratories. Thus, all students, technicians, faculty, and custodial staff working in a particular building have the same key. Keys are handed out by the facilities building representative. A student receives a key to the laboratory building if a faculty member completes a Key Authorization Form for that individual.

Bioscience Quad

The Bioscience Quad is the central focal point for all of RNU's bioscience and biomedical programs. The medical school, teaching hospital and clinic, veterinary school, animal hospital and clinic, and the biosciences department all reside on this quad. The teaching hospital and the animal hospital each house several small clinical laboratories. The main biosciences department building is principally classrooms, faculty offices, and some laboratories (for non-infectious studies). All of the department's work with infectious substances is currently conducted in the Infectious Lifesciences Laboratory. The campus incinerator is located behind the barns on the outskirts of the Quad. The Quad also has a centralized shipping and receiving facility. A cafeteria is located on the first floor of the administration building.

Infectious Lifesciences Laboratory

Facilities

ILL currently has BSL2 laboratories and BSL3 laboratories in the Sabin Research Laboratory Building (which is part of the ILL facility). Currently, ILL does not have the capacity for animal work at BSL3. The BSL3 has one pass-through autoclave, and there are several small autoclaves in individual labs. The ILL Enteric Pathogens group has one of the BSL2 laboratories for its research efforts. ILL has recently secured funding to build a new Containment Laboratory Complex. This new laboratory will greatly expand their BSL2 and BSL3 research space; it will also have dedicated ABSL2 and ABSL3 space.

ILL Staff

The following staff have access to the BSL2 and BSL3 laboratory space: faculty, research technicians, graduate students, undergraduate students (not allowed in the BSL3 laboratory), housekeeping, facility staff, and the biorisk officer.

Current Research Efforts Underway at ILL

Bacillus anthracis

PCR-based detection methods are being developed for use in identifying virulence genes specific to *B. anthracis* to facilitate the identification of *B. anthracis* from other closely related species. Faculty in the Biosciences Department and Medical School are collaborating to study the use of anthrax toxin as cancer chemotherapy.

Bovine Spongiform Encephalopathy (BSE) Prion

BSE research is a multi-disciplinary activity at ILL with research groups from physics, chemistry, biology, and computing working together to understand the mechanisms of BSE protein misfolding. ILL faculty are also interested in understanding the progression of prion diseases in livestock.

Chlamydia psittaci

Chlamydiae are obligate intracellular bacteria that occupy a non-acidified vacuole (the inclusion) throughout their developmental cycle. Little is known about events leading to the establishment and maintenance of the chlamydial inclusion membrane. Researchers are trying to identify chlamydial proteins unique to the intracellular phase of the *Chlamydia psittaci* life cycle. The ultimate objective of the *Chlamydia psittaci* research is the development of vaccines against chlamydial infections. Real-time PCR is used to detect and quantify chlamydial DNA in efforts to identify vaccine candidates.

Cryptosporidium parvum

Current research efforts are focused on studying the molecular pathogenesis of *Cryptosporidium parvum*, including host cell recognition, attachment, and invasion. The research team is also interested in improved methods for detection of the oocysts in water.

H5N1 Avian Influenza

Research on H5N1 at RNU has been limited to providing assistance to the Ministry of Agriculture in the testing of clinical samples. Positive samples have been sent to the closest World Health Organization Influenza Collaborating Center for confirmation. Pending completion of the new ILL Containment Laboratory Complex, the faculty propose to study the efficacy of oseltamivir therapy in ferrets.

***Mycobacterium tuberculosis* and MDR-TB**

Researchers seek to develop tools for improved diagnostics of *M. tuberculosis* and monitoring disease progression of drug-resistant tuberculosis. Efforts are aimed at developing immunological reagents for early diagnosis and disease monitoring. Current projects utilize green fluorescent proteins.

Rabies Virus

Researchers are studying how the rabies virus crosses the blood-brain barrier, where it is most lethal. The team is also exploring why the T and B effector cells of the immune system do not cross the barrier, and thus cannot clear the infection.

Salmonella enterica (serotype typhi)

Researchers are investigating the distribution of drug resistance in *S. typhi* across the Republic of Prowazekii. Clinics from throughout the country send isolates to the ILL Enteric Pathogens Group. The Group is testing the isolates for susceptibility to ciprofloxacin, chloramphenicol, and tetracycline. This is a long-term study to examine the change in distribution of drug resistant strains that began a decade ago.

***Shigella dysenteriae* serotype 1**

There is no vaccine available for *Shigella spp.,* and only limited treatment options for infection with antibiotic resistant strains. ILL researchers are pursing studies that will provide insights into the development of an attenuated strain suitable for use a vaccine.

Agents in Repository Collection

All agents used in current research efforts plus:

Burkholderia mallei

Eastern Equine Encephalitis Virus

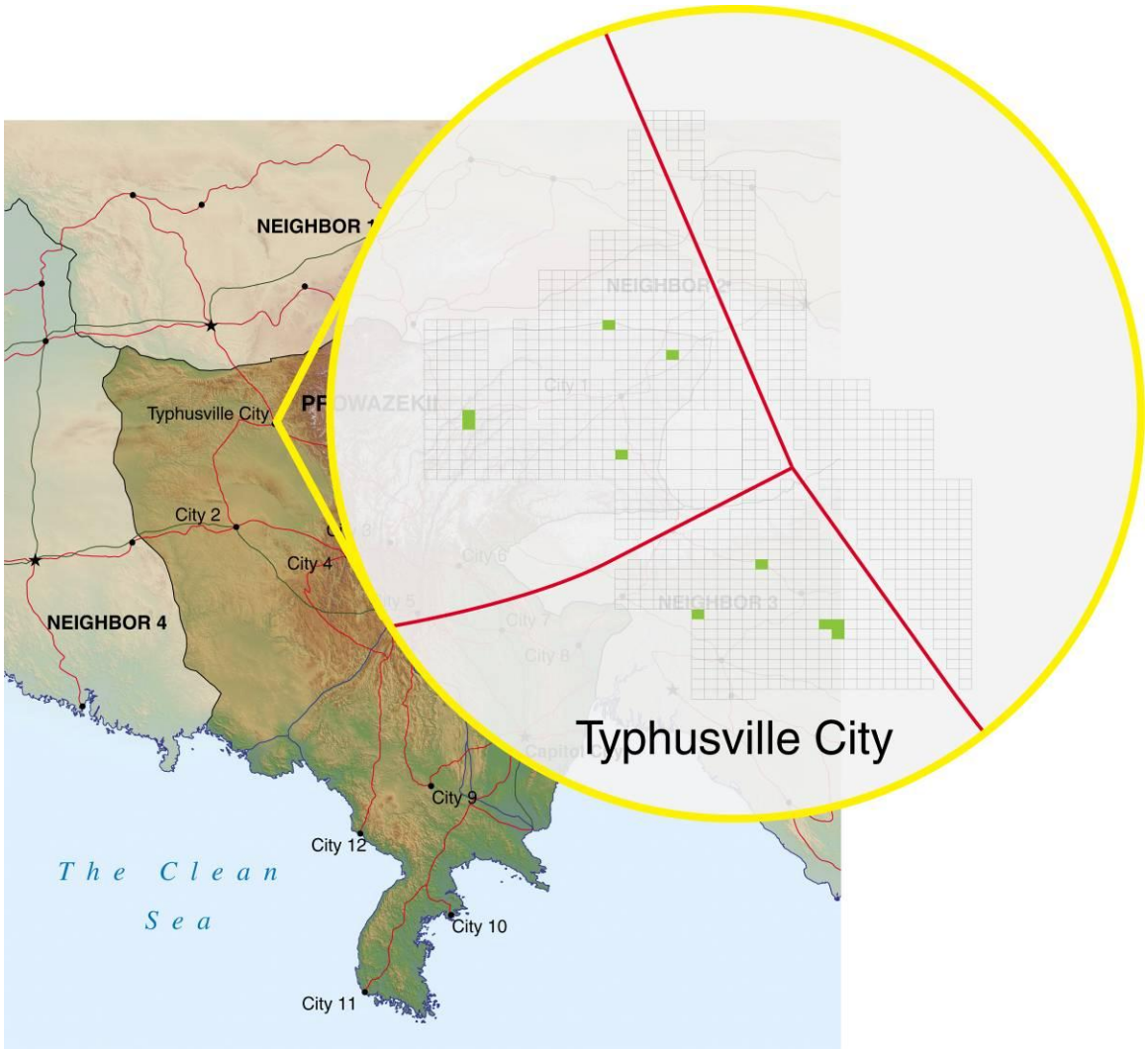
Yersinia pestis

Botulinum toxins A and F

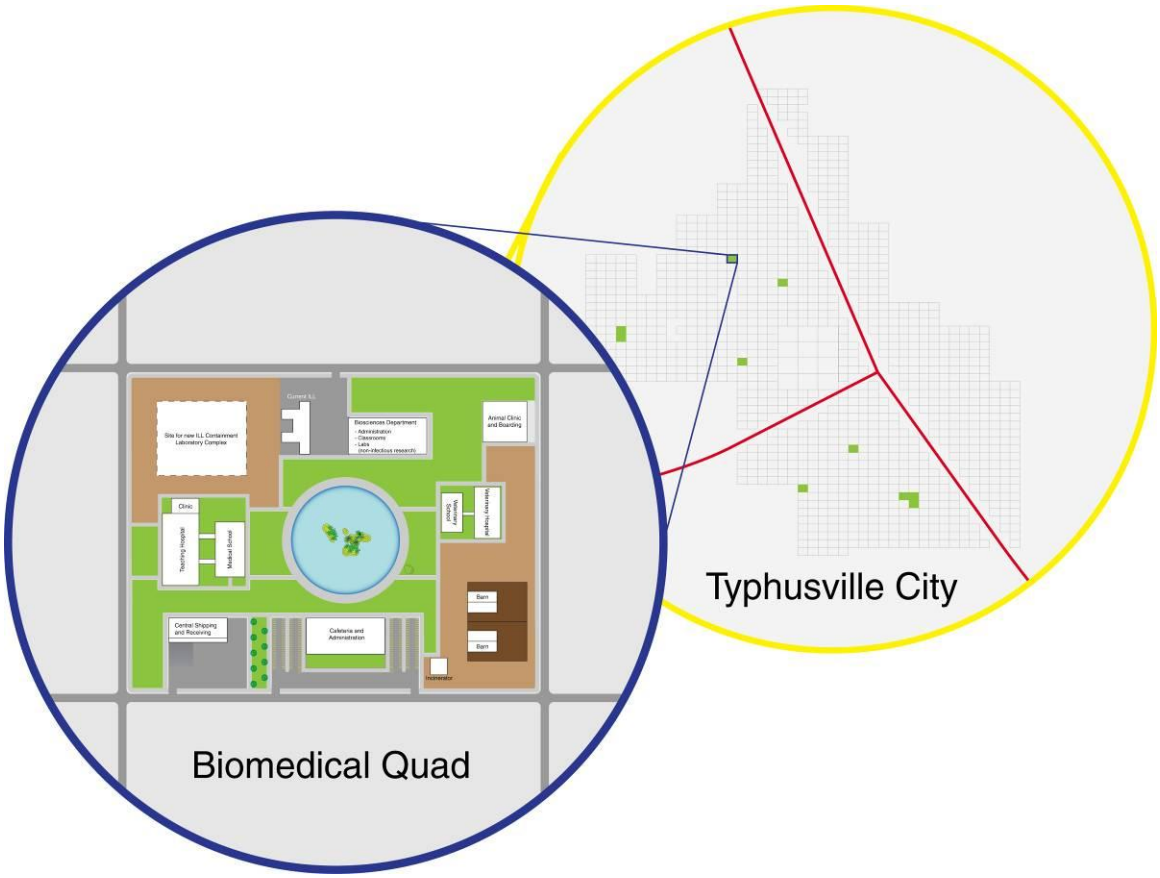
Maps

Republic of Prowazekii Map

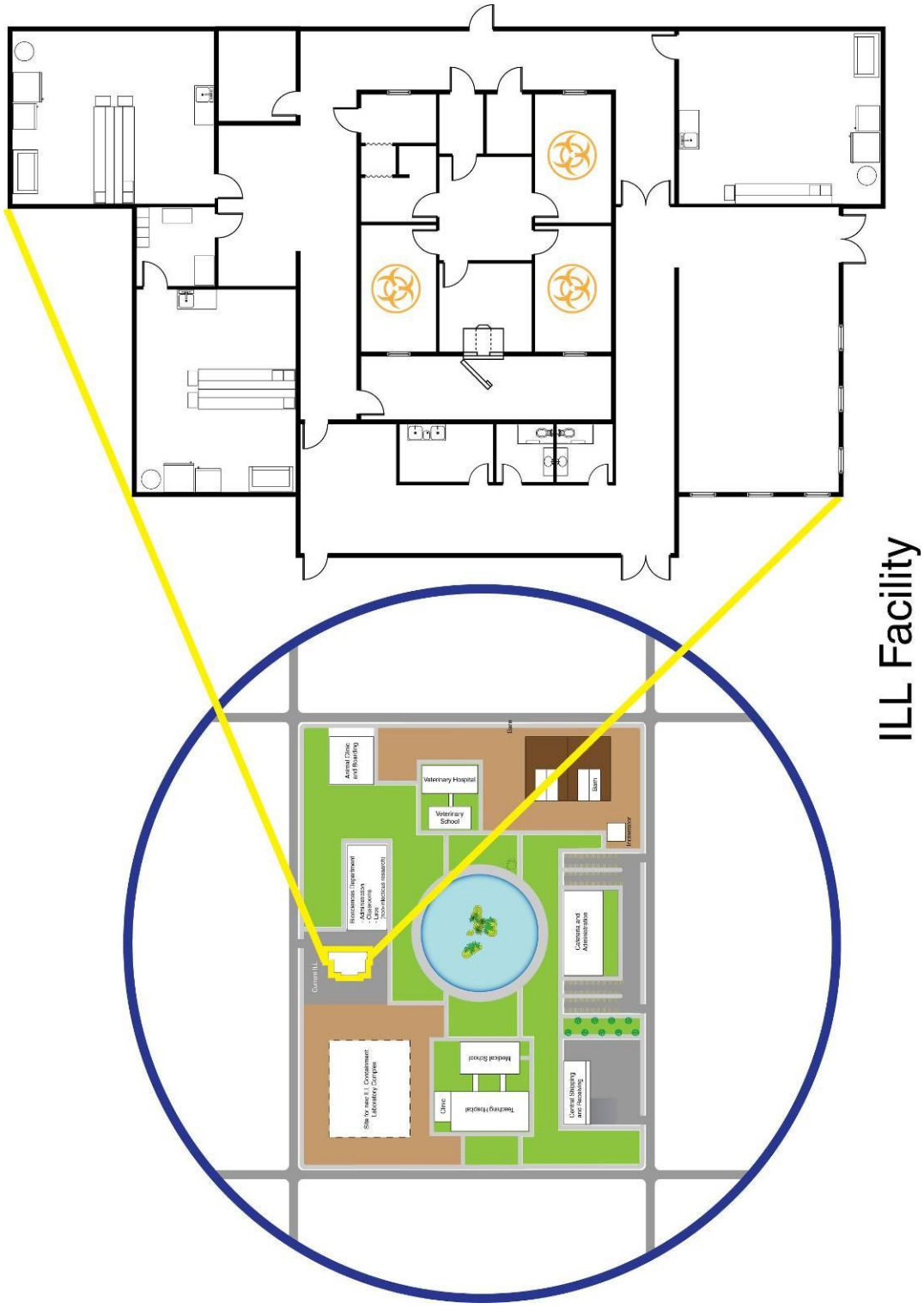
Typhusville City Map



Map of RNU's Biosciences Quad



ILL's Sabin Research Laboratory Building



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9. West emergency exit 2
10. Break area
11. Utility closet
12. Men's restroom
13. Women's restroom
14. Autoclave access area
15. Lab 4 (BSL II)
16. Dressing room
17. Anteroom
Lab 1 (BSL III) Callouts
A. Airlock
B. Mechanical room
C. Lab 1a
D. Lab 1b
E. Autoclave
F. Lab 1c
G. Change room
H. Shower
I. Entry
J. Central area

Protocol Registration Forms

In the next pages, you will find Protocol Registration Forms submitted to the Rickettsia National University's Facility Biorisk Committee for the Infectious Lifesciences Laboratory.

Incubator

Infectious Lifesciences Laboratory
Typhusville City, Republic of Prowazekii

Protocol Registration
Facility Biorisk Committee (FBC)

A protocol registration is required to ensure research conducted with biological materials is consistent with regulatory requirements, Rickettsia National University (RNU) policy, and prudent safe and secure practices for working with these materials. Registration is required for all work involving the following biohazardous agents:

- rDNA/RNA molecules in living cells
- Viable microbial agents
- Biological toxins
- Group 2, 3, or 4 biological agents as identified by the Republic of Prowazekii's Biosecurity Act
- Human or non-human primate samples or materials (including cell lines)

Complete all sections applicable to the work proposed. Once completed, submit electronically to the ILL Biorisk Officer at biorisk@ill.rnu.edu.rp. A hard copy, signed by the PI, is required in addition to the electronic submission.

Title of Protocol/Project Identifying virulence genes specific to *Bacillus anthracis*		
Principle Investigator Richard Ettsia	Phone # 11-2347-68	Mail Stop Code 1100
Location of Project/Protocol: Building/Room(s) ILL/ Lab 3		
Status of this project/protocol: ☒ New ☐ Renewal ☐ Continuation/Addendum for Project #		
Original Title if different from title above:		
Applicable SOP Title: Bacillus SOP	Approval Date: 11 June 2008	

Principal Investigator's Assurance

I am familiar with and agree to abide by the provisions of the current World Health Organization's Laboratory Biosafety Manual. I accept responsibility for this work and for monitoring the safe and work practice of the staff involved. I agree to comply with all National, Local, and RNU requirements pertaining to the shipment, transfer, storage and disposal of biohazardous materials. I will notify the committee of any significant changes to the protocol prior to institution. I certify that the information provided in this document is accurate.

_____

Signature
Principal Investigator: Date:

Personnel Involved			
Names of all personnel (including PI) that will handle the agents:	Experience (Provide a summary of the employee's experience with these agents.)	Biosafety Training Completed	BBP Training Completed (if required)
Richard Ettsia, PI	Dr. Ettsia has worked with *Bacillus* species for more than 25 years, including 20 years as PI at RNU and at Sandia.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher 1	Researcher 1 has worked with *Bacillus subtilis* in graduate school and during her 2-yr tenure as a postdoctoral fellow at RNU.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher 2	Researcher 2 is new to RNU but has worked with *Bacillus cerus* and *B. thuringiensis* for 10 years at a private company.	Yes ☐ No ☒	Yes ☐ No ☐
Technician 1	Technician 1 has worked with *Bacillus anthracis* and *B. subtilis* for the last 5 years in Dr. Ettsia's laboratory at RNU.	Yes ☒ No ☐	Yes ☐ No ☐
Student 1	Student 1 is a third year undergraduate student in Biology at RNU. She has no research experience but has had laboratory experience through coursework. She will be assisting with the culture of the *Bacillus* species to be used in this protocol. She will be assisted and supervised primarily by Researcher 2.	Yes ☒ No ☐	Yes ☐ No ☐

Protocol Type(s) - check all applicable		
Does this study involve microorganisms, viruses, or toxins?	Yes ☒ No ☐	If yes, complete **Section A**
Does this study involve viable human or non-human primate materials (e.g. blood, tissues, and cell lines)?	Yes ☐ No ☒	If yes, complete **Section B**
Does this study involve recombinant DNA or RNA molecules?	Yes ☒ No ☐	If yes, additional review is required.
Does this study involve a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act?	Yes ☒ No ☐	If yes, complete **Section A**
Is this a modification of an approved protocol?	Yes ☐ No ☐	

Abstract

Provide an abstract describing the procedures undertaken that involve the use of bioagent(s). Orient your discussion to the safety aspects of the research rather than the worthiness of the research undertaken. Include details of manipulations where appropriate. Emphasize the approach(s) that will be used to keep these agents contained within your laboratory. Address medical surveillance. Discuss any special training that will be required. Word your abstract in lay terms, whenever possible, to enhance understanding by non-scientific reviewers.

The differences between virulent and avirulent forms of a bacterial pathogen typically lie in a small set of genes that encode proteins called "virulence factors". The great success of recent genome and comparative genome sequencing efforts, together with powerful bioinformatics approaches, suggest that virulence factors will soon be identified for a broad range of human pathogens. The long-term goal of this research is to identify virulence factors that may be targeted for the design of effective vaccines and therapeutics for *Bacillus anthracis*. Experimental approaches will include assays for toxin activity for target virulence factors (including anthrax toxin 1) and quantitative real time PCR to measure expression of the virulence factors under different growth conditions. The primary goal of this work is to characterize anthrax toxin 1 (AT1) and assess the in vivo expression of anthrax toxin 1 using quantitative real time PCR. The initial studies will use PCR amplification of DNA and mRNA from *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus anthracis*. Radionucleotides (^{32}P dCTP) will be used during PCR amplification to label the DNA.

Anti-toxin approaches that treat infection therapeutically by targeting key virulence factors should provide an excellent alternative or complement to antibiotics and vaccines. Inhibition of the anthrax toxin receptor 1 (ATR1) has recently been shown to inhibit angiogenesis and tumor growth. As a result it is anticipated that inhibitors identified using the proposed assay will be valuable therapeutics against angiogenesis-dependant diseases such as cancer, macular degeneration, diabetic retinopathy, psoriasis, arthritis, and cardiovascular and cerebrovascular disease

Section A Microorganisms, Viruses, and Toxins

Type of Organism:
☒ Bacteria ☐ Fungi ☐ Virus ☐ Parasite ☐ Rickettsia ☐ Other:

Provide genus, species, and strain (if applicable): *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus anthracis*

Toxins or sub units of toxin to be used: Anthrax toxin 1

Toxin is derived from organism ☒ Toxin is of recombinant origin ☐

Source(s) of the biological material: American Type Culture Collection

Indicate which location(s) previously identified where you plan to handle this biological material: ILL / Lab 3

Indicate location where biological material will be stored: ILL / Room 2 (Freezer room)

Permit required for organism? Import Permit: Yes ☐ No ☒ Approval Date:

Copy of approved permit must be provided to Biorisk Officer on receipt.

Maximum concentration (titer) or quantity expected to have on hand at any time: 1 Liter

Is this organism or toxin regulated as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☒ No ☐

Is this organism a strain that is specifically exempted as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☐ No ☒ N/A ☐

Is a vaccine available for this organism or toxin? Yes ☒ No ☐
Is vaccination recommended? Yes ☐ No ☒
If vaccination is recommended, please specify vaccine name:
Has vaccination been offered to individuals with potential exposure? Yes ☐ No ☐

Section B Use of Viable Human or Non-Human Primate Samples or Materials

Does this project involve the use of:
Human: ☐ blood ☐ body fluids ☐ unfixed tissues ☐ other, specify
Source:

Non-human primate: ☐ blood ☐ body fluids ☐ unfixed tissues ☐ other, specify
Source:

Does this project involve the use of:
☐ Human cell culture ☐ Continuous ☐ Primary
Source:

☐ Non-human cell culture ☐ Continuous ☐ Primary
Source:

Centrifuge

Infectious Lifesciences Laboratory
Typhusville City, Republic of Prowazekii

Protocol Registration
Facility Biorisk Committee (FBC)

A protocol registration is required to ensure research conducted with biological materials is consistent with regulatory requirements, Rickettsia National University (RNU) policy, and prudent safe and secure practices for working with these materials. Registration is required for all work involving the following biohazardous agents:

- rDNA/RNA molecules in living cells
- Viable microbial agents
- Biological toxins
- Group 2, 3, or 4 biological agents as identified by the Republic of Prowazekii's Biosecurity Act
- Human or non-human primate samples or materials (including cell lines)

Complete all sections applicable to the work proposed. Once completed, submit electronically to the ILL Biorisk Officer at biorisk@ill.rnu.edu.rp. A hard copy, signed by the PI, is required in addition to the electronic submission.

Title of Protocol/Project Charactization of prion pathogenicity		
Principle Investigator Dr. Crypt Osporidium	Phone # 11-2347-55	Mail Stop Code 1125
Location of Project/Protocol: Building/Room(s) ILL/ Lab 1a		
Status of this project/protocol: ☒ New ☐ Renewal ☐ Continuation/Addendum for Project #		
Original Title if different from title above:		
Applicable SOP Title: Prion SOP	Approval Date: 03 May 2008	

Principal Investigator's Assurance

I am familiar with and agree to abide by the provisions of the current World Health Organization's Laboratory Biosafety Manual. I accept responsibility for this work and for monitoring the safe and work practice of the staff involved. I agree to comply with all National, Local, and RNU requirements pertaining to the shipment, transfer, storage and disposal of biohazardous materials. I will notify the committee of any significant changes to the protocol prior to institution. I certify that the information provided in this document is accurate.

_____

Signature
Principal Investigator: Date:

Personnel Involved			
Names of all personnel (including PI) that will handle the agents:	Experience (Provide a summary of the employee's experience with these agents.)	Biosafety Training Completed	BBP Training Completed (if required)
Dr. Osporidium, PI	The PI has over 10 years of experience working with viruses and mammalian cells and is a principal investigator in the Virology Group.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher A	Researcher A is trained as a pathogenic bacteriologist with 3 years experience at BSL2 and BSL3.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher B	Researcher B has a PhD in virology, 12 years experience working with recombinant DNA molecules, and 7 years conducting research.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher C	Researcher C has a PhD in toxinology and 5 years experience working at BSL2.	Yes ☒ No ☐	Yes ☐ No ☐
Technician A	Technician A is a biologist with experience working in a BSL2 laboratory.	Yes ☒ No ☐	Yes ☐ No ☐

Protocol Type(s) - check all applicable		
Does this study involve microorganisms, viruses, or toxins?	Yes ☒ No ☐	If yes, complete **Section A**
Does this study involve viable human or non-human primate materials (e.g. blood, tissues, and cell lines)?	Yes ☐ No ☒	If yes, complete **Section B**
Does this study involve recombinant DNA or RNA molecules?	Yes ☒ No ☐	If yes, additional review is required.
Does this study involve a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act?	Yes ☒ No ☐	If yes, complete **Section A**
Is this a modification of an approved protocol?	Yes ☐ No ☒	

Abstract

Provide an abstract describing the procedures undertaken that involve the use of bioagent(s). Orient your discussion to the safety aspects of the research rather than the worthiness of the research undertaken. Include details of manipulations where appropriate. Emphasize the approach(s) that will be used to keep these agents contained within your laboratory. Address medical surveillance. Discuss any special training that will be required. Word your abstract in lay terms, whenever possible, to enhance understanding by non-scientific reviewers.

Prion diseases are rare, fatal, neurodegenerative diseases that result either from somatic mutation or potentially the consumption of prion contaminated animal products. Prion diseases result from the conformational conversion of a normally folded, nonpathogenic prion protein to an abnormally folded, pathogenic form. Conformational change is directed by pathogenic prions exerting effects on normal, nonpathogenic prions, which stack to form filaments that eventually result in cell death. The molecular cause of cell death and neurodegeneration is not understood. However, it is thought that this results in part by the inhibition of a proteasome.

This is a proposal to investigate prion pathogenesis by mutational analysis. Deletion mutations and point mutations will be generated and analyzed using murine neuronal cell lines. In addition to analysis of mutants, we will overexpress the native prion protein to investigate neurotoxicity and proteasome inactivation.

Procedures to be used during this research include:
Cell culture for prion propagation; SDS-PAGE, Native-PAGE, and immunoblot analysis; activity probes for proteasome analysis

Section A Microorganisms, Viruses, and Toxins

Type of Organism:
☐ Bacteria ☐ Fungi ☐ Virus ☐ Parasite ☐ Rickettsia ☒ Other: prions

Provide genus, species, and strain (if applicable):

Toxins or sub units of toxin to be used: Prions

Toxin is derived from organism ☐ Toxin is of recombinant origin ☐

Source(s) of the biological material: Clinical specimens from collaborating hospitals throughout the Republic of Prowazekii.

Indicate which location(s) previously identified where you plan to handle this biological material: ILL / Lab 1a

Indicate location where biological material will be stored: ILL / Room 2 (Freezer room)

Permit required for organism? Import Permit: Yes ☐ No ☒ Approval Date:

Copy of approved permit must be provided to Biorisk Officer on receipt.

Maximum concentration (titer) or quantity expected to have on hand at any time: 3 mls. as frozen stocks

Is this organism or toxin regulated as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☒ No ☐

Is this organism a strain that is specifically exempted as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☐ No ☒ N/A ☐

Is a vaccine available for this organism or toxin? Yes ☐ No ☒
Is vaccination recommended? Yes ☐ No ☒
If vaccination is recommended, please specify vaccine name:
Has vaccination been offered to individuals with potential exposure? Yes ☐ No ☐

Section B Use of Viable Human or Non-Human Primate Samples or Materials

Does this project involve the use of:
Human: ☒ blood ☐ body fluids ☐ unfixed tissues ☒ other, specify: fecal
Source: Collaborating hospitals in the Republic of Prowazekii

Non-human primate: ☐ blood ☐ body fluids ☐ unfixed tissues ☐ other, specify
Source:

Does this project involve the use of:
☐ Human cell culture ☐ Continuous ☐ Primary
Source:

☒ Non-human cell culture ☒ Continuous ☐ Primary
Source:

Refrigerator

Infectious Lifesciences Laboratory
Typhusville City, Republic of Prowazekii

Protocol Registration
Facility Biorisk Committee (FBC)

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- rDNA/RNA molecules in living cells
- Viable microbial agents
- Biological toxins
- Group 2, 3, or 4 biological agents as identified by the Republic of Prowazekii's Biosecurity Act
- Human or non-human primate samples or materials (including cell lines)

Complete all sections applicable to the work proposed. Once completed, submit electronically to the ILL Biorisk Officer at biorisk@ill.rnu.edu.rp. A hard copy, signed by the PI, is required in addition to the electronic submission.

Title of Protocol/Project Characterization of glycoprotein of rabies virus ERA and the role in pathogenesis		
Principle Investigator Dr. Babesia	Phone # 11-2347-54	Mail Stop Code 1125
Location of Project/Protocol: Building/Room(s) ILL/ Lab 4		
Status of this project/protocol: ☒ New ☐ Renewal ☐ Continuation/Addendum for Project #		
Original Title if different from title above:		
Applicable SOP Title: Rabies virus SOP	Approval Date: 03 May 2008	

Principal Investigator's Assurance

I am familiar with and agree to abide by the provisions of the current World Health Organization's Laboratory Biosafety Manual. I accept responsibility for this work and for monitoring the safe and work practice of the staff involved. I agree to comply with all National, Local, and RNU requirements pertaining to the shipment, transfer, storage and disposal of biohazardous materials. I will notify the committee of any significant changes to the protocol prior to institution. I certify that the information provided in this document is accurate.

_____

Signature
Principal Investigator: Date:

Personnel Involved			
Names of all personnel (including PI) that will handle the agents:	Experience (Provide a summary of the employee's experience with these agents.)	Biosafety Training Completed	BBP Training Completed (if required)
Dr. Babesia, PI	The PI has over 20 years of experience working with viruses and mammalian cells and is a principal investigator in the Virology Group.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher A	Researcher A is trained as a pathogenic bacteriologist with experience at BSL2 and BSL3.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher B	Researcher B has a PhD in virology, 25 years experience working with recombinant DNA molecules, and 12 years conducting research at BSL2 and BSL3.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher C	Researcher C has a PhD in Biochemistry and has worked as a virologist in BSL2 labs for 18 years.	Yes ☒ No ☐	Yes ☐ No ☐
Technician A	Technician A is a biologist with experience working in a BSL2 laboratory.	Yes ☒ No ☐	Yes ☐ No ☐

Protocol Type(s) - check all applicable		
Does this study involve microorganisms, viruses, or toxins?	Yes ☒ No ☐	If yes, complete **Section A**
Does this study involve viable human or non-human primate materials (e.g. blood, tissues, and cell lines)?	Yes ☒ No ☐	If yes, complete **Section B**
Does this study involve recombinant DNA or RNA molecules?	Yes ☒ No ☐	If yes, additional review is required.
Does this study involve a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act?	Yes ☒ No ☐	If yes, complete **Section A**
Is this a modification of an approved protocol?	Yes ☐ No ☒	

Abstract

Provide an abstract describing the procedures undertaken that involve the use of bioagent(s). Orient your discussion to the safety aspects of the research rather than the worthiness of the research undertaken. Include details of manipulations where appropriate. Emphasize the approach(s) that will be used to keep these agents contained within your laboratory. Address medical surveillance. Discuss any special training that will be required. Word your abstract in lay terms, whenever possible, to enhance understanding by non-scientific reviewers.

Rabies virus (RV) is the causative agent of a fatal encephalomyelitis in many mammalian species, including humans. The RV is comprised of a negative stranded, nonsegmented RNA genome that is approximately 12 kb in length. Five gene products are encoded; the glycoprotein is the only protein exposed to the surface of the mature virion and is the major contributor to pathogenicity of particular RV. Glycoproteins of pathogenic RV strains have been shown to accelerate virus internalization into host cells.

This is a proposal to further characterize the function of RV glycoprotein in pathogenesis, and elucidate its role in host cell internalization and spread. These objectives will be achieved by generation of chimeric recombinant viruses; the glycoprotein genes from attenuated RV strains (SN) will be exchanged for those in highly virulent strains (SB). Pathogenesis will be evaluated by observing growth kinetics in cell culture. Efficient spread of the resulting constructs will be evaluated using Vero cells. All mutants will be compared to wild type strains isolated from clinical specimens.

Methods to be used for this study include the following:
Viral titer determination by immunoflourescence and growth curves; cloning; western blot analysis; RNA analysis using quantitative PCR

Section A Microorganisms, Viruses, and Toxins

Type of Organism:
☐ Bacteria ☐ Fungi ☒ Virus ☐ Parasite ☐ Rickettsia ☐ Other:

Provide genus, species, and strain (if applicable): Rabies virus strain SN (attenuated); RV strain SB (virulent)

Toxins or sub units of toxin to be used: None

Toxin is derived from organism ☐ Toxin is of recombinant origin ☐

Source(s) of the biological material: Clinical specimens from collaborating veterinary hospitals and facilities throughout the Republic of Prowazekii and culture collection.

Indicate which location(s) previously identified where you plan to handle this biological material: ILL / Lab 4

Indicate location where biological material will be stored: ILL / Room 2 (Freezer room)

Permit required for organism? Import Permit: Yes ☐ No ☒ Approval Date:

Copy of approved permit must be provided to Biorisk Officer on receipt.

Maximum concentration (titer) or quantity expected to have on hand at any time: 3 mls. as frozen stocks

Is this organism or toxin regulated as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☒ No ☐

Is this organism a strain that is specifically exempted as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☐ No ☒ N/A ☐

Is a vaccine available for this organism or toxin? Yes ☒ No ☐
Is vaccination recommended? Yes ☒ No ☐
If vaccination is recommended, please specify vaccine name: Rabies Vaccine for Human Use
Has vaccination been offered to individuals with potential exposure? Yes ☒ No ☐

Section B Use of Viable Human or Non-Human Primate Samples or Materials

Does this project involve the use of:
Human: ☐ blood ☐ body fluids ☐ unfixed tissues ☒ other, specify: brain
Source: Collaborating veterinary hospitals and facilities in the Republic of Prowazekii

Non-human primate: ☐ blood ☐ body fluids ☐ unfixed tissues ☐ other, specify
Source:

Does this project involve the use of:
☐ Human cell culture ☐ Continuous ☐ Primary
Source:

☐ Non-human cell culture ☒ Continuous ☐ Primary
Source: Culture collection

Storage

Infectious Lifesciences Laboratory
Typhusville City, Republic of Prowazekii

Protocol Registration
Facility Biorisk Committee (FBC)

A protocol registration is required to ensure research conducted with biological materials is consistent with regulatory requirements, Rickettsia National University (RNU) policy, and prudent safe and secure practices for working with these materials. Registration is required for all work involving the following biohazardous agents:

- rDNA/RNA molecules in living cells
- Viable microbial agents
- Biological toxins
- Group 2, 3, or 4 biological agents as identified by the Republic of Prowazekii's Biosecurity Act
- Human or non-human primate samples or materials (including cell lines)

Complete all sections applicable to the work proposed. Once completed, submit electronically to the ILL Biorisk Officer at biorisk@ill.rnu.edu.rp. A hard copy, signed by the PI, is required in addition to the electronic submission.

Title of Protocol/Project Understanding the Distribution of Drug Resistance in *Salmonella typhi* across the Republic of Prowazekii		
Principle Investigator Cory Nebacterium	Phone # 11-2347-52	Mail Stop Code 1125
Location of Project/Protocol: Building/Room(s) ILL/ Lab 1a		
Status of this project/protocol: ☐ New ☒ Renewal ☐ Continuation/Addendum for Project #		
Original Title if different from title above:		
Applicable SOP Title: *Salmonella* SOP	Approval Date: 03 May 2008	

Principal Investigator's Assurance

I am familiar with and agree to abide by the provisions of the current World Health Organization's Laboratory Biosafety Manual. I accept responsibility for this work and for monitoring the safe and work practice of the staff involved. I agree to comply with all National, Local, and RNU requirements pertaining to the shipment, transfer, storage and disposal of biohazardous materials. I will notify the committee of any significant changes to the protocol prior to institution. I certify that the information provided in this document is accurate.

_____

Signature

Principal Investigator: Date:

Personnel Involved			
Names of all personnel (including PI) that will handle the agents:	Experience (Provide a summary of the employee's experience with these agents.)	Biosafety Training Completed	BBP Training Completed (if required)
Cory Nebacterium, PI	The PI has over 20 years of experience working with bacteria, viruses and mammalian cells and is head of the Enteric Pathogens Group.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher A	Researcher A is trained as a pathogenic bacteriologist with experience at BSL2 and BSL3.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher B	Researcher B has a PhD in pathogenic bacteriology, 8 years experience working with recombinant DNA molecules, and 3 years conducting research at BSL2 and BSL3.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher C	Researcher C has a PhD in Immunology and will be trained to handle pathogens.	Yes ☒ No ☐	Yes ☐ No ☐
Technician A	Technician A is a biologist with experience working in a BSL2 laboratory.	Yes ☒ No ☐	Yes ☐ No ☐

Protocol Type(s) - check all applicable		
Does this study involve microorganisms, viruses, or toxins?	Yes ☒ No ☐	If yes, complete **Section A**
Does this study involve viable human or non-human primate materials (e.g. blood, tissues, and cell lines)?	Yes ☐ No ☒	If yes, complete **Section B**
Does this study involve recombinant DNA or RNA molecules?	Yes ☒ No ☐	If yes, additional review is required.
Does this study involve a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act?	Yes ☒ No ☐	If yes, complete **Section A**
Is this a modification of an approved protocol?	Yes ☐ No ☒	

Abstract

Provide an abstract describing the procedures undertaken that involve the use of bioagent(s). Orient your discussion to the safety aspects of the research rather than the worthiness of the research undertaken. Include details of manipulations where appropriate. Emphasize the approach(s) that will be used to keep these agents contained within your laboratory. Address medical surveillance. Discuss any special training that will be required. Word your abstract in lay terms, whenever possible, to enhance understanding by non-scientific reviewers.

This is a proposal to continue our investigation of the distribution of drug resistance in *Salmonella typhi* across the Republic of Prowazekii. Clinics from throughout the country send isolates to the Enteric Pathogens Group. This Group plans to test the isolates for susceptibility to ciprofloxacin, chloramphenicol, and tetracycline. This is a long-term study that began ten years ago.

Occurrence of *S. typhi* in the clinical isolates will be confirmed by enrichment culture technique in selenite F broth followed by plating on Salmonella Shigella agar. The identity of the cultures will be subsequently confirmed by serotyping and PCR detection of virulence genes (inv A and spv C). The Kirby-Bauer disc diffusion technique will be used to test for drug-resistance.

Section A Microorganisms, Viruses, and Toxins

Type of Organism:
☒ Bacteria ☐ Fungi ☐ Virus ☐ Parasite ☐ Rickettsia ☐ Other:

Provide genus, species, and strain (if applicable): *Salmonella enterica (serotype typhi)*

Toxins or sub units of toxin to be used: None

Toxin is derived from organism ☐ Toxin is of recombinant origin ☐

Source(s) of the biological material: Clinical specimens from collaborating hospitals throughout the Republic of Prowazekii.

Indicate which location(s) previously identified where you plan to handle this biological material: ILL / Lab 1a

Indicate location where biological material will be stored: ILL / Room 2 (Freezer room)

Permit required for organism? Import Permit: Yes ☐ No ☒ Approval Date:

Copy of approved permit must be provided to Biorisk Officer on receipt.

Maximum concentration (titer) or quantity expected to have on hand at any time: 3 mls. as frozen stocks

Is this organism or toxin regulated as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☒ No ☐

Is this organism a strain that is specifically exempted as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☐ No ☒ N/A ☐

Is a vaccine available for this organism or toxin? Yes ☐ No ☒
Is vaccination recommended? Yes ☐ No ☒
If vaccination is recommended, please specify vaccine name:
Has vaccination been offered to individuals with potential exposure? Yes ☐ No ☐

Section B Use of Viable Human or Non-Human Primate Samples or Materials

Does this project involve the use of:
Human: ☒ blood ☐ body fluids ☐ unfixed tissues ☒ other, specify: fecal
Source: Collaborating hospitals in the Republic of Prowazekii

Non-human primate: ☐ blood ☐ body fluids ☐ unfixed tissues ☐ other, specify
Source:

Does this project involve the use of:
☐ Human cell culture ☐ Continuous ☐ Primary
Source:

☐ Non-human cell culture ☐ Continuous ☐ Primary
Source:

Storage

Infectious Lifesciences Laboratory
Typhusville City, Republic of Prowazekii

Protocol Registration
Facility Biorisk Committee (FBC)

A protocol registration is required to ensure research conducted with biological materials is consistent with regulatory requirements, Rickettsia National University (RNU) policy, and prudent safe and secure practices for working with these materials. Registration is required for all work involving the following biohazardous agents:

- rDNA/RNA molecules in living cells
- Viable microbial agents
- Biological toxins
- Group 2, 3, or 4 biological agents as identified by the Republic of Prowazekii's Biosecurity Act
- Human or non-human primate samples or materials (including cell lines)

Complete all sections applicable to the work proposed. Once completed, submit electronically to the ILL Biorisk Officer at biorisk@ill.rnu.edu.rp. A hard copy, signed by the PI, is required in addition to the electronic submission.

Title of Protocol/Project Characterization of Osp F and OspC1 deletion mutants of *Shigella dysenteria*		
Principle Investigator Dr. Borrelia	Phone # 11-2347-53	Mail Stop Code 1125
Location of Project/Protocol: Building/Room(s) ILL/ Lab 2		
Status of this project/protocol: ☒ New ☐ Renewal ☐ Continuation/Addendum for Project #		
Original Title if different from title above:		
Applicable SOP Title: *Shigella* SOP	Approval Date: 03 May 2008	

Principal Investigator's Assurance

I am familiar with and agree to abide by the provisions of the current World Health Organization's Laboratory Biosafety Manual. I accept responsibility for this work and for monitoring the safe and work practice of the staff involved. I agree to comply with all National, Local, and RNU requirements pertaining to the shipment, transfer, storage and disposal of biohazardous materials. I will notify the committee of any significant changes to the protocol prior to institution. I certify that the information provided in this document is accurate.

_____

Signature
Principal Investigator: Date:

Personnel Involved			
Names of all personnel (including PI) that will handle the agents:	Experience (Provide a summary of the employee's experience with these agents.)	Biosafety Training Completed	BBP Training Completed (if required)
Dr. Borrelia, PI	The PI has 5 years of experience working with bacteria and mammalian cells, and is a principal investigator in the Enteric Pathogens Group.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher A	Researcher A is trained as a pathogenic bacteriologist with experience at BSL2.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher B	Researcher B has a PhD in biochemistry, 20 years experience working with recombinant DNA molecules, and 10 years conducting research at BSL2.	Yes ☒ No ☐	Yes ☐ No ☐
Researcher C	Researcher C has a PhD in biochemistry and has worked as a pathogenic microbiologist in BSL2 labs for 2 years.	Yes ☒ No ☐	Yes ☐ No ☐
Technician A	Technician A is a biologist with experience working in a BSL2 laboratory.	Yes ☒ No ☐	Yes ☐ No ☐

Protocol Type(s) - check all applicable		
Does this study involve microorganisms, viruses, or toxins?	Yes ☒ No ☐	If yes, complete **Section A**
Does this study involve viable human or non-human primate materials (e.g. blood, tissues, and cell lines)?	Yes ☒ No ☐	If yes, complete **Section B**
Does this study involve recombinant DNA or RNA molecules?	Yes ☒ No ☐	If yes, additional review is required.
Does this study involve a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act?	Yes ☐ No ☒	If yes, complete **Section A**
Is this a modification of an approved protocol?	Yes ☐ No ☒	

Abstract

Provide an abstract describing the procedures undertaken that involve the use of bioagent(s). Orient your discussion to the safety aspects of the research rather than the worthiness of the research undertaken. Include details of manipulations where appropriate. Emphasize the approach(s) that will be used to keep these agents contained within your laboratory. Address medical surveillance. Discuss any special training that will be required. Word your abstract in lay terms, whenever possible, to enhance understanding by non-scientific reviewers.

Shigella dysenteria is Gram negative bacteria that causes severe diarrheal disease in humans. Though the disease is usually self-limiting, it frequently results in the death of children in third world and developing countries. Pathogenesis is multi-faceted, requiring numerous concerted activities, initiated by invasion of the organism into a susceptible host. One facet of pathogenesis is the formation of a Type Three Secretion System (T3SS) necessary to deliver secreted effector molecules to the host cells. Osp F and Osp C1 gene products are secreted by the organism, but their functions are unknown.

This is a proposal to elucidate the functions of these two effector molecules secreted by the T3SS, identify their role in pathogenesis, and determine their location within host cells. The approach will be to generate deletion mutants and point mutations in each gene individually and simultaneously. Mutants will be characterized by their ability to infect confluent HeLa cell monolayers by plaque formation assays. The location of Osp F and Osp C1 gene products in the host cell will be determined by immunofluorescence by generating 2-Hemagglutinin (2HA) tagged gene products and anti-2HA antibodies. All mutants will be compared to wild type strains and other strains isolated from clinical specimens.

Section A Microorganisms, Viruses, and Toxins
Type of Organism: ☒ Bacteria ☐ Fungi ☐ Virus ☐ Parasite ☐ Rickettsia ☐ Other:
Provide genus, species, and strain (if applicable): *Shigella dysenteria*
Toxins or sub units of toxin to be used: None Toxin is derived from organism ☐ Toxin is of recombinant origin ☐
Source(s) of the biological material: Clinical specimens from collaborating hospitals throughout the Republic of Prowazekii.
Indicate which location(s) previously identified where you plan to handle this biological material: ILL / Lab 1a Indicate location where biological material will be stored: ILL / Room 2 (Freezer room)
Permit required for organism? Import Permit: Yes ☐ No ☒ Approval Date: Copy of approved permit must be provided to Biorisk Officer on receipt.
Maximum concentration (titer) or quantity expected to have on hand at any time: 3 mls. as frozen stocks
Is this organism or toxin regulated as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☐ No ☒ Is this organism a strain that is specifically exempted as a Group 2, 3, or 4 biological agent as identified by the Republic of Prowazekii's Biosecurity Act? Yes ☐ No ☐ N/A ☒
Is a vaccine available for this organism or toxin? Yes ☐ No ☒ Is vaccination recommended? Yes ☐ No ☒ If vaccination is recommended, please specify vaccine name: Has vaccination been offered to individuals with potential exposure? Yes ☐ No ☐
Section B Use of Viable Human or Non-Human Primate Samples or Materials
Does this project involve the use of: Human: ☐ blood ☐ body fluids ☐ unfixed tissues ☒ other, specify: fecal Source: Collaborating hospitals in the Republic of Prowazekii Non-human primate: ☐ blood ☐ body fluids ☐ unfixed tissues ☐ other, specify Source:
Does this project involve the use of: ☐ Human cell culture ☐ Continuous ☐ Primary Source: ☐ Non-human cell culture ☐ Continuous ☐ Primary Source:

Case Study: Biorisk Assessment Class Activity

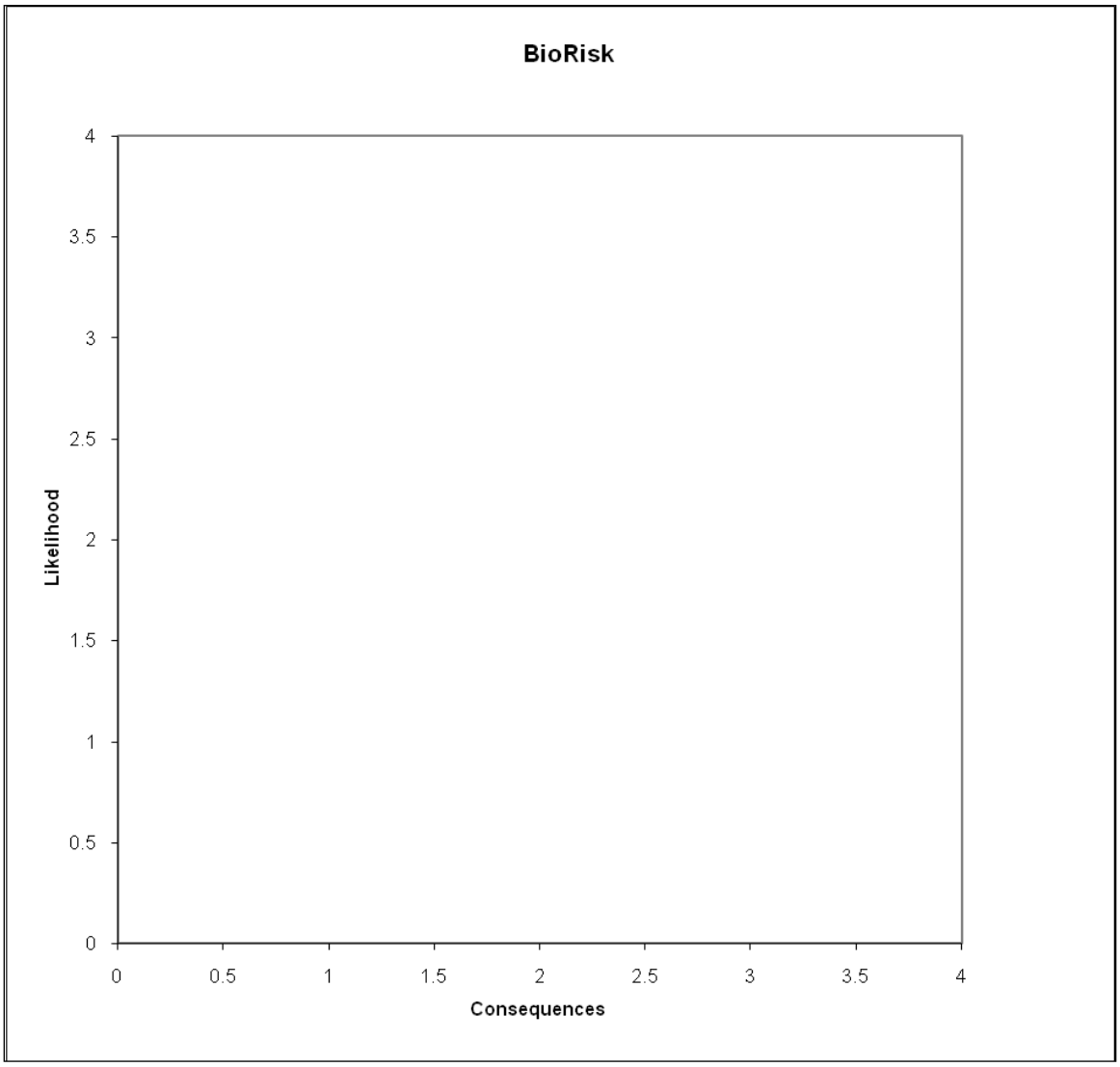
Conduct a risk assessment based upon your case study.

Define the risks: What risks do you need to assess (consider safety and security risks)?

Use the board to characterize these risks (plot the likelihood and the consequences). Use the blank graph on the next page as well.

Explain why these risks have been characterized the way they have.
Eg. “There is a high likelihood of infection given that the agent is infectious via inhalation.”

Is this risk acceptable? Why or why not?


BioRisk
Likelihood
Consequences
0
0.5
1
1.5
2
2.5
3
3.5
4

Case Study: Personal Protective Equipment

Determine what PPE is the most appropriate for the agent and the activity in your case study. Why did you choose the PPE that you did?

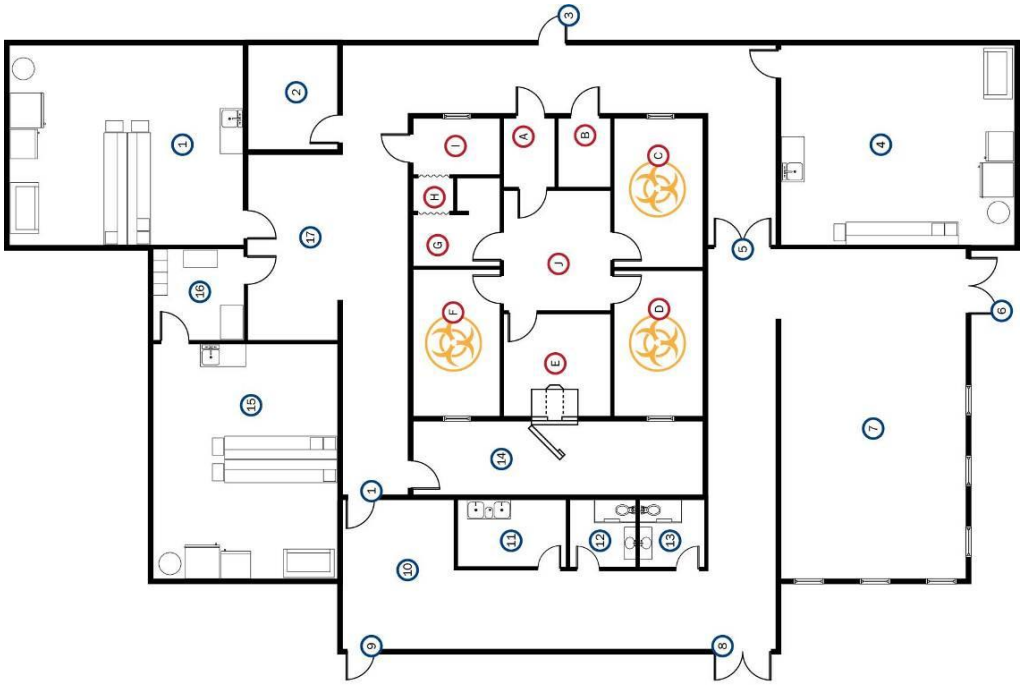
Create a donning and doffing order for the PPE that you have determined, and your lab.

- Where is the PPE stored?
- Where is the PPE disposed of or cleaned?
- Are there any other considerations?

Case Study: Good Laboratory Practices

What special “good laboratory practices” will you require for the work you are reviewing? Who will be accountable for the biological agents used in the research? What will be your inventory requirements?

Case Study: Directional Airflow from Clean to Dirty



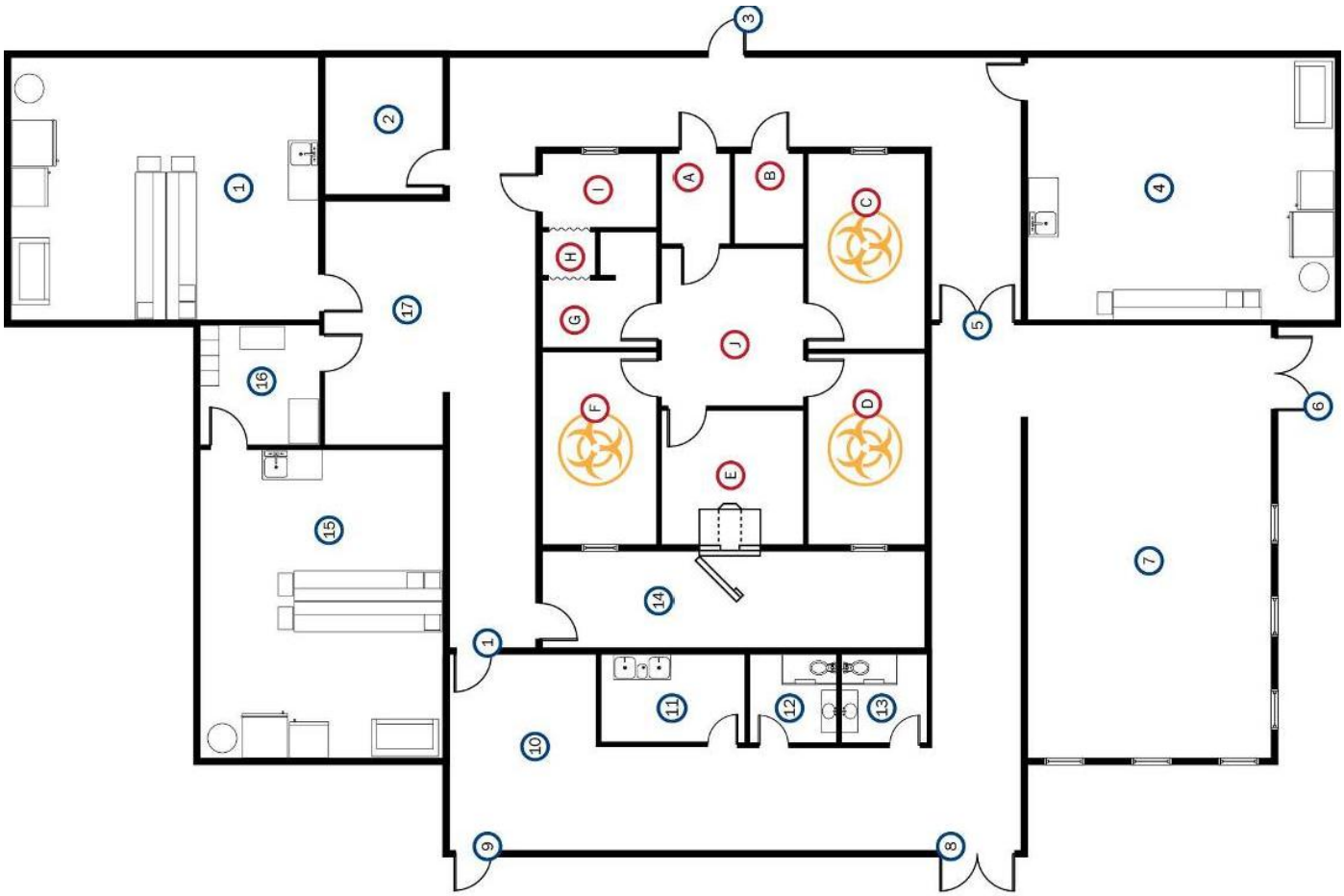
General Facility Callouts

1. Lab 2 (BSL II)
2. Freezer
3. East emergency exit
4. Lab 3 (BSL II)
5. Limited Area entrance
6. Main building entrance
7. Offices and work areas
8. Delivery entrance / West emergency exit 1
9. West emergency exit 2
10. Break area
11. Utility closet
12. Men's restroom
13. Women's restroom
14. Autoclave access area
15. Lab 4 (BSL II)
16. Dressing room
17. Anteroom

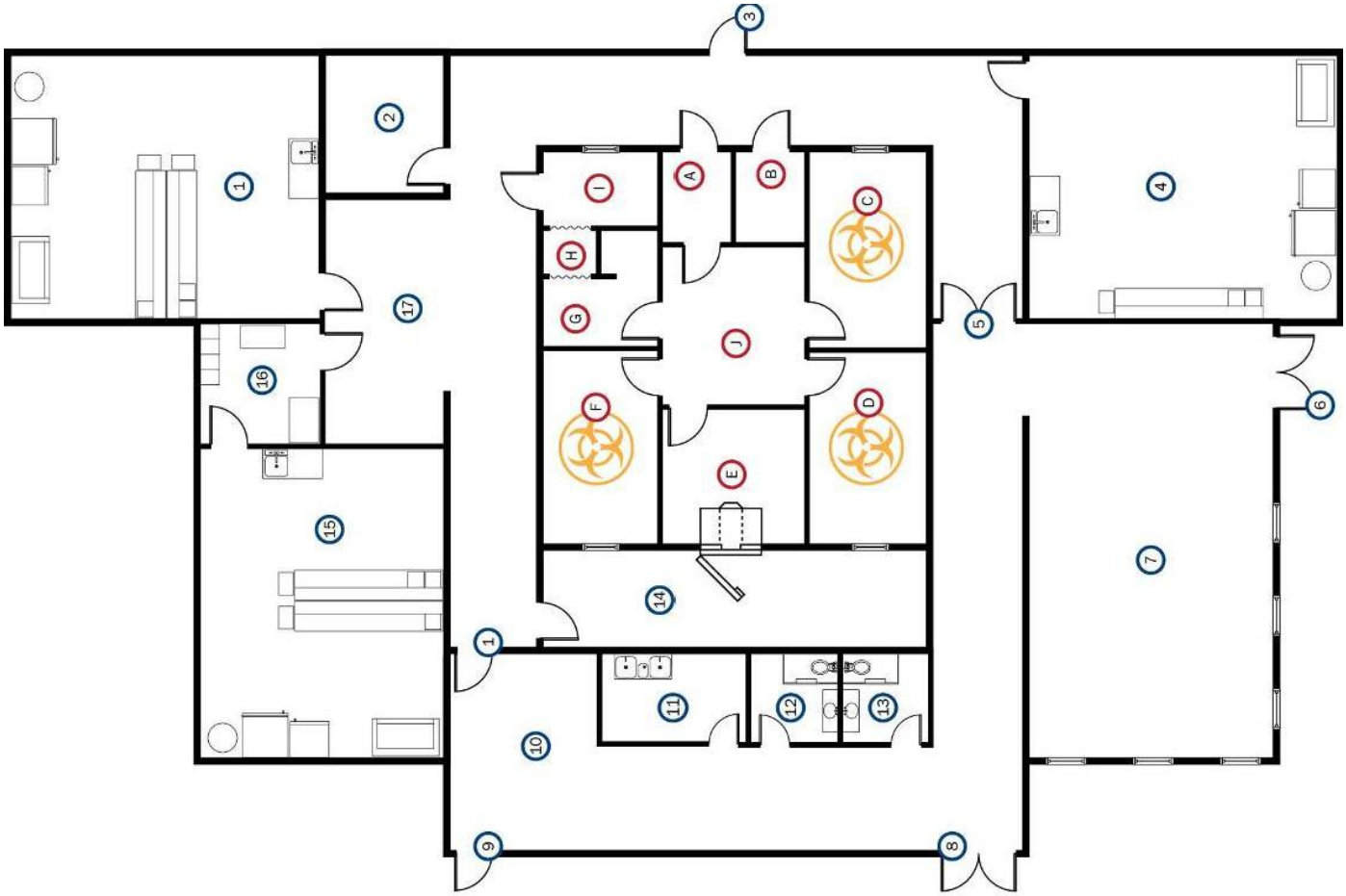
Lab 1 (BSL III) Callouts

A. Airlock
B. Mechanical room
C. Lab 1a
D. Lab 1b
E. Autoclave
F. Lab 1c
G. Change room
H. Shower
I. Entry
J. Central area

Identify the clean and dirty areas on the ILL floor plan:



Please draw arrows on the ILL floor plan to indicate the direction of airflow.



Case Study: Biological Safety Cabinet

1. For your project, what work will you require to be done in the biosafety cabinet?

2. What type of biosafety cabinets would be the best choices for the laboratories where your project research is being carried out? Please justify your choices.

Case Study: Waste Handling and Decontamination

For your research protocol, use the questions below as a guide to outlining how waste and any need for decontamination will be addressed. Please explain your answers.

1. How will solid waste be handled?

2. How will liquid waste be handled?

3. How will work surfaces be decontaminated in the event of a small spill (e.g. <20 ml)?

4. Are there any situations in which you would want the ability to do a gaseous decontamination of the lab? If so, please describe the process to decontaminate the lab.

Case Study: Incident Reporting System

Develop incident reporting system to be used for any incidents involving the work in your protocol at the hypothetical facility.

Consider the following questions:

- What are some specific, potential incidents to think about for your case study?
- Who should be notified in the event of a spill, exposure, theft, or other incident?
- What forms will be filled out?
- What is the timeframe for reporting the incident?

Case Study: Granting Laboratory Access

The Facility Biorisk Committee is tasked with determining who will have access to the ILL and how that access will be granted. Rickettsia National University policy is to have an open campus to facilitate collaboration and sharing of equipment and other expensive resources. The Committee must balance this policy with the responsibility to operate the facility safely and securely.

1. Please explain who will have access to which areas of the ILL and why.
2. How will access privileges be granted? What administrative requirements will be established?
3. Are there any medical requirements for access?
4. Will visitors be allowed into the facility? Under what conditions? Are there different types of visitors?
5. What training requirements will be required before an individual is granted access to the laboratories? Do all the laboratories have the same training requirements?
6. What happens when an individual no longer needs access?
7. How will access be granted or managed for normal system maintenance? For emergency maintenance?
8. Other considerations?

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9. West emergency exit 2
10. Break area
11. Utility closet
12. Men's restroom
13. Women's restroom
14. Autoclave access area
15. Lab 4 (BSL II)
16. Dressing room
17. Anteroom
Lab 1 (BSL III) Callouts
A. Airlock
B. Mechanical room
C. Lab 1a
D. Lab 1b
E. Autoclave
F. Lab 1c
G. Change room
H. Shower
I. Entry
J. Central area

Case Study: Designing a Physical Security System for the ILL

The Facility Biorisk Committee determine what physical security measures you will require to be implemented prior to the protocol being approved and work starting in the laboratory. You will need to present a short summary of your physical security plans in the final presentation. Please be sure that your physical security system supports the access decisions you made in the case study on granting access to the laboratory.

1. Identification of the assets
    a. Where in the laboratory space will the agents be stored and used in your protocol?

    b. Where is the isolate repository (freezer room)?

2. Define the Security System Strategy and explain why?
    - Deny
    - Contain
    - Deter

3. Design System
    a) Define any *special exclusions* and *exclusion areas*

    b) Define the *limited areas*

    c) Define the points of entry into the limited and exclusion areas

d) Define where access control systems should be used and why

e) Define where intrusion detection systems and alarms should be used and explain how alarm information should be communicated and displayed, and to whom?

f) Which individuals should be included in the review of the physical security system? With whom should MOUs be established? Do any existing MOUs need to be updated?

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10. Break area
11. Utility closet
12. Men's restroom
13. Women's restroom
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D. Lab 1b
E. Autoclave
F. Lab 1c
G. Change room
H. Shower
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Case Study: Developing a Biorisk Program Management System

Outline the elements of the Biorisk Program Management System your committee believes are important for the proposed research your committee is reviewing.

Case Study Reference: Descriptions of Notional Adversaries

Insiders

These individuals have authorized access. A facility may have only one type of insider (the Insider with Full Access) or a facility may have multiple types of insiders based on the level of access to the agent that might be stolen, such as Insider with Full Access, Insider with Building Access, and Insider with Site Access. There are some attributes that are assumed to apply to all Insider adversaries. An Insider's motive may be due to disgruntlement, psychological imbalance, personal gain (in collusion), or the desire to commit a terrorist act. The malevolent insider would be expected to abort any theft attempt in order to avoid detection because, with authorized access, the insider can wait for a more opportune time to steal the agent. In general, the insider is an employee who has become malevolent, and is not assumed to have paramilitary training.

The Insider with Full Access may be a laboratory worker or other individual who has unescorted access to the asset. Authorized access affords this person extensive knowledge of the facility and operating systems. It also provides this adversary with opportunity. Insiders with Full Access to biological materials are usually scientists and technicians who have a high level of technical training and sophistication. Thus, they often have all of the means at their disposal to successfully acquire and maliciously disseminate a biological agent.

Outsiders

These individuals do not have authorized access to the site. Information specific to the site and vicinity should be used to modify these notional descriptions or create additional ones.

Terrorist Group: The motive of this adversary is to cause mass casualties, an economic crisis, or widespread fear and/or it may seek to make a political statement. This adversary is assumed to be well-funded, and may be supported by a state, religious group, individuals, or even organized crime. Being well-funded, the Terrorist Group is also well-equipped and trained, and able to rehearse an attack. The Terrorist Group is highly organized. The Terrorist Group is violent and willing to die. The Terrorist Group also has access to, and the skill to utilize, significant explosives and arms. As an Outsider, the Terrorist Group does not have access or specific opportunity.

Single Terrorist: This adversary may be motivated to make a political statement, to express anger, to steal an agent in order to ultimately achieve personal goals, or to commit an act of bioterrorism. This adversary has less means than the Terrorist Group, but is still well-equipped and trained, and able to rehearse. He may still be capable of killing or injuring a number of guards or other individuals. The Single Terrorist has the tools necessary to overcome most access control systems. The Single Terrorist is willing to use violence and force. As an Outsider, the Single Terrorist does not have access or specific opportunity.

Extremist Group: This adversary is motivated to make a political statement or protest against programs for ecological, political, economic, or other reasons. As a result, its objective may not be theft of a biological agent; instead, it may be to destroy property or release animals. However, this group's acts may inadvertently cause an escape of pathogens into the environment by releasing contaminated animals. The Extremist Group has the ability to commit sabotage, and may possess hand tools, items for arson and other facility sabotage, and possibly handguns. The Extremist Group has general information about the facility, but not specific information about the location of the assets or the facility's protection systems. All members of the group are assumed to be Outsiders, thus the Extremist Group does not have access or specific opportunity.

Criminal: This adversary is motivated by financial gain. The Criminal is a single adversary that may have weapons and hand tools. If organized crime is assessed to be a local problem, a Criminal Group adversary can be defined with limited capabilities. The Criminal adversary is also presumed to be an outsider without access or specific opportunity.

Competitive Rival: This adversary seeks to gain competitive market advantage through theft or destruction of proprietary information or experimental materials. The Competitive Rival may include an invited colleague or other visitor who has the intent to steal intellectual property and/or acquire an agent. This adversary is assumed to have limited capabilities, but he may have opportunity with direct, supervised access (i.e., as an Escorted Insider).

Vandal: This adversary may operate individually or in groups. His motivation is to cause a nuisance by damage or destruction. His tools include spray paint, knives, hand tools, and may include guns for target shooters or hunters. A Vandal attacks local facilities is not homicidal. A vandal has no authorized access to the facility or opportunity to steal pathogens and toxins.

Colluding Terrorist Group: This is a combination of an Insider and an Outsider Terrorist Group. However, by involving more individuals, this notional adversary puts themselves at risk of discovery.

Case Study Reference: Agent Fact Sheets

Bacillus anthracis

Environmental Factors

1) Geographic Distribution:

Anthrax is endemic throughout the world; outbreaks occur sporadically. The World Anthrax Data Site categorizes 14 countries as being "hyperendemic" for anthrax, these are: Turkey, Tajikistan, Myanmar, Niger, Chad, Ethiopia, Zambia, Zimbabwe, Togo, Ghana, Cote d'Ivoire, Liberia, Sierra Leone, and Guinea – all but three are in Africa, many in West Africa. A large number of countries in Africa and Asia are considered "endemic"; very few countries are considered anthrax-free. The US has sporadic cases of disease in animals; very rarely do humans contract the disease naturally.

2) Disease Symptoms (human and animal):

Clinical manifestations vary greatly, and depend on route of infection: cutaneous, gastrointestinal, and inhalation. Cutaneous: a red macule skin infection becomes papular and vesiculated, then ulcerates and develops into a blackened, depressed eschar. Lesion is painless; surrounding lymph glands may swell; ~5-20% case fatality if untreated. Gastrointestinal: abdominal pain, vomiting, bloody diarrhea, fever, and septicemia; fatality rate 25-60% if untreated. Inhalation: initial symptoms often resemble common cold or other respiratory diseases, but often eventually results in massive bacteremia and secondary pneumonia. Respiratory distress quickly gets worse, fever develops, and shock may occur; usually fatal. In humans, ~95% of all cases are cutaneous, ~5% are inhalation, <1% are gastrointestinal. The disease is usually fatal in livestock such as cattle, sheep, goats and horses. Anthrax runs its course so rapidly that it can be difficult to diagnose in live animals; sudden death is usually a characteristic sign of disease. Symptoms often seen before death include high temperatures and swelling in the neck and shoulder areas. Swine and dogs are more resistant to the acute disease but develop extensive swelling in the neck area.

3) Strain information:

Strains found in the environment exhibit vast differences in pathogenicity and virulence; only a small percentage are very dangerous to people. Thus far, 89 genetically distinct strains of anthrax have been described. However, plasmid composition adds to the genetic variety, anthrax bacilli can carry numerous copies of two different plasmids—pX01 and pX02. Strains may carry up to 243 copies of pX01 and up to

32 copies of pX02;[2] the more copies of the latter plasmid, the more severe the disease. Strains devoid of the plasmid pX02 plasmid, such as the Sterne strain, are avirulent. However, the genetic content of the bacteria's chromosome also dictate virulence – e.g., the Ames strain which possess only 2 pX02 plasmids is very dangerous.

4) Reservoirs/Vectors:

Herbivorous animals, including cattle, sheep, goats, and pigs, are the natural reservoir. Other mammals, birds, and humans also contract the disease.

5) Agent Sources:

Soil contaminated with spores, blood and tissues from gravely ill or recently deceased animals, contaminated meat or animal products (hides, etc). Carcasses of animals that recently died of anthrax are highly infectious and excellent sources. In humans, vesicular fluid swabs in cutaneous cases, stool and blood in gastrointestinal cases, sputum and blood in inhalation cases.

6) Stability:

B. anthracis spores are extremely resistant to environmental duress including drying, heat, and sunlight; also resistant to numerous disinfectants. Spores can remain viable in soil, animal skins, hides, and wool for decades. One estimate suggests survival of 40 years or more in dry soil. The spores can survive in milk for 10 years; on filter paper for 41 years; on silk threads for 71 years; and in pond water for 2 years. 2% glutaraldehyde, formaldehyde, and 5% formalin inactivates the spores.

Laboratory Factors

1) Security Classification:

US Select Agent

Prowazekii Group 2 Agent

2) General Research Volume:

[2] Coker PR, Smith KL, Fellows PF, Rybachuck G, Kousoulas KG, Hugh-Jones ME. Bacillus anthracis virulence in guinea pigs vaccinated with anthrax vaccine adsorbed is linked to plasmid quantities and clonality. J Clin Microbiol. 2003;41:1212–1218.

Relatively large volume of research (in Pubmed)

3) Survey Information:

51/722 (7.06%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *B. anthracis*.

4) Former Weapons Activity:

A number of countries, including Canada, Germany, Iraq, Japan, the Soviet Union, the UK, and the US weaponized *B. anthracis*. France, and South Africa had conducted anthrax BW research. Iran, Syria, and North Korea are suspected of having current and active BW programs, conducting anthrax research and possibly weaponizing the agent already.

5) Culture Collections:

Anthrax is a very common disease that has affected mankind for a long time. It is estimated that a large number of culture collections located at germ banks, universities, etc. contain *B. anthracis* stocks.

6) General Growth Conditions:

Fresh blood (taken during acute stages of disease to within a few days of death, prior to antibiotic treatment), sputum, and tissue samples taken from sick or recently deceased animals (or humans) can be readily cultured. If gastrointestinal anthrax is suspected, rectal or stool samples may yield bacteria. Isolation of *B. anthracis* from soil, old carcasses, or processed specimens such as hides is more complicated. Soil surrounding a decaying carcass is likely to harbors spores. *B. anthracis* grows readily on many types of routine, commonly available media at 35-37ºC. The bacterium will grow well on sheep blood agar, MacConkey agar, and chocolate agar plates from blood, sputum, and vesicular fluid swabs. In ~24 hours, 2-5 mm nonhemolytic, grey-white colonies that are flat, round (sometimes irregular edges) will appear, often exhibiting comma-shaped projections ("Medusa head" appearance). Simple stains, microscopy, and PCR can be used to identify anthrax; polymerase chain reaction (PCR) can be used to detect plasmids necessary for virulence or small animals can be inoculated with the organism.

Safety Factors

1) Risk of Disease Contraction:

Humans can get cutaneous anthrax when spores enter the body through broken skin; gastrointestinal anthrax by ingesting spores, often in contaminated, undercooked meat; and inhalation anthrax by when spores of less that 5µm in diameter become lodged in the lower region of the lungs. Only cutaneous anthrax is transmitted human-to-human, and is extremely rare. Infectious dose estimated to be between 8,000 and 50,000 bacilli via inhalation.

2) Countermeasures (PPE and medical):

B. anthracis should be isolated and identified using BSL2 practices. Biological safety cabinets should be used to manipulate any cultures or spore suspensions. BSL3 facilities should be used for any work with spores and larger quantities of agent. PPE should include a respirator capable of filtering out spores, gloves, a laboratory coat, and face shields or goggles. Antibiotics must be administered early to be effective. Penicillin is generally the drug of choice; erythromycin, ciprofloxacin, doxycycline, and vancomycin are also effective. Many countries immunize herbivorous animals with live attenuated vaccines; human vaccines are much less common and are administered to those in high risk occupations.

Bovine Spongiform Encephalopathy Prion

Environmental Factors

1) Geographic Distribution:

Bovine spongiform encephalopathy (BSE) was first recognized in England in 1986, but cases have since been detected throughout most of Europe, more recently the disease has been detected in Asia and North America (very few cases). Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Japan, Liechtenstein, Luxembourg, the Netherlands, Poland, Portugal, Slovakia, Slovenia, Spain, and Switzerland have all reported BSE in indigenous cattle populations. However, England has accounted for >90% of cases worldwide; as of January 2004, 180,000 cases confirmed from more than 35,000 different herds. However, due to prevention measures, incidence has gone down drastically. In some countries, disease has been linked to the import of infected cattle or feed containing contaminated meat-and-bone meal from countries with BSE; in other countries BSE arose indigenously. The peak incidence is often in cattle between 4 and 5 years of age; no breed predilection.

2) Disease Symptoms (human and animal):

BSE is a disorder that causes progressive neurological degeneration in cattle; symptoms are very similar to those of scrapie, a similar neurological disease in sheep. The clinical course of BSE varies, but can extend for several months; however BSE is invariably fatal. Early clinical signs are subtle and nonspecific; later signs are somewhat variable but very distinct. Aggregation (in the form of fibrils) of a disease-specific isoform of the host-encoded Prion Protein (PrP) membrane protein (PrP^{res} prion) causes lesions in the Central Nervous System (CNS). Lesions are characterized by sponge-like changes visible with an ordinary microscope; leading to neurological defects and changes in behavior. Animals display temperamental changes such as apprehension, aggression, and hyper-reactivity. Later, abnormal posture, incoordination, weakness; animals lose weight and produce less milk. Variant Creutzfeldt-Jakob disease (vCJD) is a rare and fatal human neurodegenerative condition also caused by CNS lesions that lead to spongy degeneration of the brain. vCJDhas been strongly linked to exposure to BSE prions. Patients normally experience psychiatric symptoms early in illness, most commonly depression, sometimes schizophrenia-like psychosis. Unusual sensory symptoms, such as "stickiness" of the skin, are experienced by half of the cases early on. Neurological signs develop as illness progresses, including unsteadiness, difficulty walking and involuntary movements; before death, patients are completely immobile and mute. No diagnostic tests have been developed to detect the BSE agent in live animals.

3) Strain information:

Multiple strains or isolates of BSE exhibit different incubation periods and patterns of neuropathological change when administered to mice. For quite some time, only one BSE strain was known, however as of the beginning of 2008, 2 or more are known to exsist.

4) Reservoirs/Vectors:

The causative agent of BSE is a modified form of a genetically encoded, highly conserved bovine membrane protein.

5) Agent Sources:

BSE prion has been found in brain tissue, spinal cord, and the retina of the eye. Additional experimental studies suggest that BSE prion may also be present in the small intestine, tonsil, bone marrow, and dorsal root ganglia.

6) Stability:

Aggregates of the BSE isoform of PrP are protease resistant and very stable. The prions are extremely resistant to heat, ultraviolet light, ionizing radiation, normal sterilization processes, and common disinfectants that normally inactivate viruses and bacteria. High concentrations of either sodium hypochlorite or 2N sodium hydroxide for ~1 hour, or autoclaving at high temperatures is required for disinfection.

Laboratory Factors

1) Security Classification:

US Select Agent

Prowazekii Group 3 Agent

2) General Research Volume:

In general, large volume of research (in Pubmed)

3) Survey Information:

No survey information.

4) Former Weapons Activity:

No reports of any research for BW purposes

5) Culture Collections:

Few culture collections, research and diagnostic veterinary labs will likely have infected samples or specimens. Large veterinary reference laboratories may, but specimens will likely be in high security/safety setting due to potential agricultural damage.

6) General Growth Conditions:

Methods for diagnosis include direct examination of spongiform encephalopathy morphological features by histopathological examination of brain, observation of PrP^{res} fibrils using electron microscopy and/or immunohistochemical labeling. These methods require that the obex (a portion of the brain stem) be extracted and carefully sectioned. Rapid immunoassays have recently been developed for quick screening, but must be used on the brain tissue of slaughtered animals – can't screen live animals. The presence of the BSE prion and overall level of infectivity is assessed by injecting animals (mostly mice) with brain tissue homogenates, and then observing symptoms. These tests may take up to 700 days. Brain tissue should be removed quickly after slaughter. There are no *in vitro* methods for isolation of BSE agent (no culture system). Currently, only animal models such as mice and macaques can be used to "grow" the agent. Portions of infected brains containing PrP^{res} are homogenated in glucose solution (20% v/v (volume) if intracerebrally administered, 2% if intraperitoneally). Animals can also be fed infected material.

Safety Factors

1) Risk of Disease Contraction:

The chief cause of BSE in cattle is the ingestion of food tainted with prion contaminated meat-and-bone meal added as a supplement. Experimentally, BSE prion can also be transmitted by parental and oral exposure to infected bovine brain tissue; however BSE is not a contagious disease. Evidence suggests that exposure to the BSE agent causes the vCJD in humans, possibly by ingesting contaminated beef. No scientific evidence to suggest that milk and dairy products carry the BSE agent.

2) Countermeasures (PPE and medical):

Materials contaminated with the causative agent of BSE should be handled in BSL3 facilities, but there is no evidence that agent can be aerosolized, so HEPA filtration is not necessary. Penetrating injuries, contamination of abraded skin, and ingestion should be avoided; use of standard surgical masks, gowns, and disposable gloves will minimize the risk of working with BSE. In event of infection, there is no known treatment or cure.

Burkholderia mallei

Environmental Factors

1) Geographic Distribution:

Burkholderia Mallei has been eliminated largely from most regions of the world; it is still endemic in enzootic foci in some Middle Eastern countries (Turkey, Syria, Iraq, Iran), South Asia (Pakistan and the Indian subcontinent), Southeast Asia (Burma, Indonesia, Philippines), parts of China and Mongolia, and Africa. The Balkan states and former Soviet republics may also still have *B. mallei*. Glanders is sporadic in Europe and the Americas.

2) Disease Symptoms (human and animal):

Three forms of illness, depending on route of infection: chronic pulmonary form (more common is horses), Farcy, an acute septicemic form (more common in mules and donkeys). And Glanders, which typically results in formation of nodules and ulcers in the lungs, and subcutaneous vesicles filled with exudates in mucous membranes, particularly in nostrils, which rupture into ulcers; submaxillary lymph nodes are generally firm. Equids develop characteristic greyish-yellow viscid discharge from nostrils (frequent snorting due to impeded breathing). As disease progresses, ulcers on the nose increase in number, enlarge or become confluent, extend in depth and sometimes completely perforate the septum; nasal discharge streaked with blood. Acute illness in equids includes a high fever, weight loss, and respiratory signs like breathing difficulties, pneumonia, cough, and swollen nostrils; ulcers form in lungs; death typically occurs quickly. Chronic disease includes pulmonary symptoms and cutaneous symptoms, but symptoms much more mild; horses may survive several years, becoming carriers. Farcy is more of a local infection where multiple nodules form in the skin and ulcerate, discharging yellow, oily pus. In humans, common clinical signs include fever, myalgia, headaches, chest pain, sensitivity to light, excess tear production, diarrhea, and muscle tightness.

3) Strain information:

A large number of strains likely exist, but little information in literature; an older study (1947) showed great differences in infectiousness between laboratory strains.

4) Reservoirs/Vectors:

Equids, particularly horses, mules, and donkeys are reservoirs. Humans are accidental hosts; can also infect camels, dogs, and cats.

5) Agent Sources:

In live animals: wound/nodule exudates or blood samples (collected only during first 3 days infection); at necropsy: exudates from nasal passage and the upper respiratory tract. Bacteria are numerous in smears from fresh lesions; few present in older lesions. It may also be possible to culture sputum and urine, although this will be less effective.

6) Stability:

The bacterium is not an environmental pathogen and its main reservoir is animals; it can remain active for up to six weeks in horse stables. It can survive and remain virulent up to 30 days in water at room temperature. The agent is deactivated by many disinfectants: 1% sodium hypochlorite, 70% ethanol, and 2% glutaraldehyde, and sensitive to desiccation, UV irradiation, and heat greater than 55 °C.

Laboratory Factors

1) Security Classification:

US Select Agent

Prowazekii Group 2 Agent

2) General Research Volume:

Generally rather low volume of research (in Pubmed)

3) Survey Information:

31/722 (4.29%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *B. mallei*.

4) Former Weapons Activity:

Germany used rudimentary techniques to disseminate *B. mallei* during WWI; Japan weaponized and used *B. mallei* during WWII. The Soviet Union also weaponized *B. mallei*; the US conducted BW research.

5) Culture Collections:

Research conducted on *B. mallei* for long time, but less so now that it has been eradicated in many places. However, old stocks likely exist in many research/clinical labs worldwide.

6) General Growth Conditions:

Best to attempt isolation from unopened, uncontaminated lesions; bacteria can be first identified in stained smears taken from exudates. Growth is slow, but can be improved with enrichment of glycerol. Optimal growth at 37°C in aerobic conditions on ordinary culture media supplemented with 1-5% glucose and/or 5% glycerol enrichment media. After approx. 48 hours, bacteria visible as confluent, smooth, moist growth, that is viscid and somewhat creamy in color. Longer incubation results in thick growth that is dark brown and tough. Bacteria also grows well on glycerol potato agar and in glycerol broth; a selective media (nutrient agar containing 4% glycerine + polymyxin B, bacitracin, actidione, donkey/horse serum, tryptone agar) has been developed for isolation from contaminated samples. Guinea pigs very susceptible; used to test for virulent bacteria. Enzyme-Linked ImmunoSorbent Assay's (ELISA) are often used in diagnostic testing; PCR more so recently.

Safety Factors

1) Risk of Disease Contraction:

Humans contract the disease mainly from direct contact with nasal and skin secretions of infected equines; discharge is extremely infectious. Bacteria enter body through compromised skin and mucus membranes of eyes, nose, and mouth; so transmission is possible by the routes of ingestion, inhalation of aerosols, and direct or indirect contact with skin lesions and abrasions. Human-to-human transmission of *B. mallei* is possible but unlikely. Handling lab cultures is a high risk activity.

2) Countermeasures (PPE and medical):

Any laboratory work with infectious body fluids, tissues, or cultures should be confined to BSL3 facilities. Respirators should be worn to prevent inhalation of aerosolized bacteria; gowns, gloves, and eye protection (preferably face shields) should be used to prevent any contact with infectious droplets. If working with infected animals, puncture-resistant Kevlar gloves should be worn. Severe disease must be treated aggressively with multiple systemic antibiotics: most strains sensitive to antibiotics such as ceftazidime, imipenem, doxycycline, minocycline, ciprofloxacin, and gentamycin, but treatment may take a long time. Strains are often resistant to tetracyclines. Vaccines are unavailable.

Chlamydia psittaci

Environmental Factors

1) Geographic Distribution:

Worldwide, sporadic disease; natural incidence is unknown. The disease is often associated with pet birds, particularly psittacine birds in pet shops and aviaries. Humans in high risk occupations—e.g. on turkey or duck farms—often contract the disease. Likely 50-200 cases/year in US, but incidence can vary greatly from year to year.

2) Disease Symptoms (human and animal):

In birds, clinical signs may vary significantly according to host species, age of infected birds, and bacterial strain. Generally the disease is acute, with signs: conjunctivitis, loss of appetite and weight loss, ruffled feathers, nasal secretions and sneezing, diarrhea and yellowish, sometimes bloody stools. Pneumonia may follow. Often, birds (especially older psittacine birds) will exhibit no symptoms but will continue to shed the agent for long period; the disease is usually quite severe in ducks. In humans: presentation can vary from inapparent to acute, systemic disease, often with fever, chills, headache, photophobia, respiratory symptoms, often interstitial pneumonia, cough, and myalgia. Encephalitis is possible. Clinical pictures ranges from asymptomatic to severe, often lethal atypical pneumonias with multi-organ failure. In other mammals: mostly pneumonia and abortion.

3) Strain information:

Strains are distributed among 6 known avian serovars (A-F) and two mammalian serovars; the mammalian strains do not appear to circulate among birds and very few outbreaks have involved these strains. Serovar D strains (largely in turkeys) appear to cause particularly high morbidity and mortality in birds; very severe disease in humans. When the pathogen is transmitted from psittacine birds to humans it is considered particularly virulent to the human host, but all strains considered transmissible to humans.

4) Reservoirs/Vectors:

Birds, particularly psittacine (parrot family) birds, are natural reservoir; but the organisms have been detected in over 130 bird species (57 psittacine). Many different birds, including parakeets, parrots, pigeons, turkeys, ducks, and ostriches can carry the agent; many of these birds may be asymptomatic but continue to shed the pathogen. Animals and humans are also susceptible.

5) Agent Sources:

Feces and nasal discharge of infected birds; may also be present is blood, tissues, and eggs from infected birds. In acute cases, nasal discharge, blood, and tissue samples may be used; if diarrhea, excrement and cloacal swabs can be used. Infected birds may shed the pathogen intermittently or continuously for several weeks or even months. The blood, sputum, and tissues of infected humans carry the agent.

6) Stability:

C. psittaci elementary bodies are capable of surviving for months in environment under the right conditions and are very resistant to drying. The bacteria can survive for 52 hours in infected egg fluids, a few days in bird droppings, 2 months in bird feed, for 15 days on glass, and 20 days in straw. The bacteria may survive for up to a year in turkey carcasses. The agent is susceptible to many disinfectants including 1% sodium hypochlorite, 70% ethanol, glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

Prowazekii Group 1 Agent

2) General Research Volume:

Some laboratory based work—moderate to high amount (in Pubmed); sometimes grown in reference laboratories

3) Survey Information:

35/722 (4.85%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *C. psittaci*.

4) Former Weapons Activity:

The Soviets conducted research on *C. psittaci*, as did the US.

5) Culture Collections:

Probably in small number of legitimate culture collections, would expect some reference labs to have samples.

6) General Growth Conditions:

The isolation and identification of the agent is used to diagnose disease definitively. Isolation may be achieved by inoculating animals, chicken embryos, or, most conveniently, cell lines (typically buffalo green monkey (BGM), Vero, McCoy, HeLa, or L cells); contaminant bacteria may sometimes make *C. psittaci* isolation difficult but pretreatment with antibiotics can help. Samples homogenized in antibiotic solution and supernatant used for inoculation. Infection of cells is enhanced by centrifuging (500-1500g for 30-90 minutes at 37°C) the inoculum onto the cell monolayer; addition of cyclohemimide and other cell division inhibitors also enhances infectivity. The organism is grown in McCoy cells at 38°C in monlayer cultures in Eagle's minimum essential medium containing 10% fetal calf serum (MEM) and antibiotics non-inhibitory to C. psittaci. Protocols are available for growth and isolation in chicken embryos. Cell cultures should be observed for several days using a variety of staining methods: Gimenz, Giemsa, Ziehl-Neelson, Machiavello's. Fluorescent antibodies can also be used to detect bacteria in culture. Serovar-specific antibodies and PCR methods have been developed to serotype isolates.

Safety Factors

1) Risk of Disease Contraction:

Transmission to humans is normally through contact with the excretions of infected animals; principally by inhalation of dessicated droppings and secretions of infected birds. The bite of an infected bird can also transmit the disease. Diseased birds that are asymptomatic (often psittacines and pigeons) are the most important source of human infection. Transmission from infected ducks and turkeys to humans occurs frequently during slaughter. Person-to-person transmission is rare, but could occur through droplet production during coughing. *C. psittaci* is one of the most commonly reported laboratory-acquired infections.

2) Countermeasures (PPE and medical):

BSL2 containment and practices are recommended for handling of potentially contaminated material, and necropsy of infected birds. Particular care must be applied when handling dead infected birds, as the feathers may harbor residual infectious feces and nasal secretions. For laboratory activities that result in large volumes of agent, or have an increased potential to produce droplets or aerosols, BSL3

containment and practices should be employed. Laboratory coats and gloves should be worn whenever handling infectious material to prevent any contact with skin; important to use respiratory protection for any work with infected live birds. A number of antibiotics are effective against *C. psittaci*, doxycycline is often the favored antibiotic; tetracycline, erythromycin, azithromycin, and clarithromycin are also used but are not always effective; a number of isolates have been resistant to penicillin.

Clostridium botulinum

Environmental Factors

1) Geographic Distribution:

The *C. botulinum* spores are widely distributed in nature in anaerobic environments (soil, water, aquatic sediments, etc.). Spores may be found in soil, in water (streams, lakes, coastal waters) and aquatic sediments, in the gastrointestinal tracts of fish and mammals, and in the gills and viscera of mollusks and shellfish. Disease in humans occurs sporadically (often rarely) worldwide, often in association with the consumption of contaminated foods. Infant botulism most common form in US (serotype A more common west of Mississippi; serotype B east).

2) Disease Symptoms (human and animal):

There are three forms of botulism: foodborne, wound, and infant; all are generally characterized by the same set of symptoms. Botulism is characterized by acute flaccid paralysis. Initially individuals experience paralysis of the facial and neck muscles, often experiencing double or blurred vision, drooping eyelids, slurred speech, difficulty swallowing, dry mouth, and muscle weakness. Eventually paralysis may occur in the arms, legs, and respiratory muscles; and death results from respiratory failure. The neurological signs are sometimes preceded by nausea, abdominal cramps, vomiting, or diarrhea in foodborne botulism. Infant botulism occurs principally in infants under one year of age; clinical severity can vary greatly.

3) Strain information:

A number of genetically diverse toxigenic strains of *Clostridium* species can produce neurotoxin. Seven serotypes (A through G) of *C. botulinum* are recognized, based on the antigenic specificity of the toxin produced by each strain. Serotypes A, B, and E principally cause human botulism (occasionally F). The World Health Organization (WHO) reports that the current mortality rate is 5% (type B) to 10% (type A). Serotypes C and D cause most cases of botulism in mammals and birds. Although type G has been isolated from soil in Argentina, no outbreaks involving it have been recognized.

4) Reservoirs/Vectors:

C. botulinum is widely distributed in nature in soil, water, and aquatic sediments. Bacteria can also inhabit the intestinal tract of animals; in humans, bacteria can generally only colonize the intestinal tracts of infants and the immuno-compromised. Bacteria may contaminate food and agricultural

products if sterilization procedures are poor. Animals most commonly affected are wild fowl and poultry, cattle, horses and some species of fish.

5) Agent Sources:

Environmental samples such as soil and water may harbor the organism. Proper human specimens include feces, enema fluid, gastric aspirates or vomitus, tissue or exudates, and postmortem specimens. The feces of animals with botulism may also harbor bacteria. Improperly prepared canned or bottled food products may also harbor toxin-producing bacteria.

6) Stability:

Spores found in the environment are extremely resilient, capable of surviving up to 2 hours at 100°C. However, spores are destroyed after 15 minutes in moist heat at 120°C; toxin is destroyed after boiling for 10 minutes. Bacteria are susceptible to a large number of disinfectants, including 1% sodium hypochlorite, and 70% ethanol; botulinum toxin inactivated by 0.1% sodium hypochlorite, or 0.1N NaOH.

Laboratory Factors

1) Security Classification:

US Select Agent (labs with over 0.5 mg must abide by requirements—registration, etc.)

Prowazekii Group 3 Agent

2) General Research Volume:

Relatively large volume of research (in Pubmed)

3) Survey Information:

49/722 (6.79%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *C. botulinum*. 42/722 (5.82%) contain botulinum toxins.

4) Former Weapons Activity:

The US program first produced botulinum toxin during World War II; the toxin was eventually weaponized. The Former Soviet Union also produced large quantities of botulinum toxin for weapons use. Iraq also weaponized botulinum toxin, and used it to arm a number of missiles and bombs. Canada, France, South Africa, and the UK conducted BW research on the agent. Currently, Iran, North Korea, and Syria are believed to have either already developed or are in the process of developing botulinum toxin as a weapon. Aum Shinrikyo apparently isolated the *C. botulinum* strain they used in their attacks from soil samples collected in northern Japan.

5) Culture Collections:

Long history of research and global presence suggests numerous culture collections, and diagnostic/clinical labs have this agent.

6) General Growth Conditions:

Feces can be suspended in PBS, diluted, and plated directly on solid media. Treatment with heat (80ºC) or ethanol can select against non-spore forming bacteria. *C. botulinum* will grow well when directly inoculated onto commercially available anaerobe blood agar or phenylethyl alcohol blood agar (PEA); Brucella agar (with 5% sheep blood), Columbia agar, or brain heart infusion agar (with yeast extract, vitamin K, and hemin). Cultures should be incubated for 1-2 days at 35-37ºC under anaerobic conditions. Colonies are grey-white with circular/irregular edges and are β-hemolytic. The agent can also be enriched in certain broth cultures; broth cultures that are grown under optimal conditions for toxin production may contain up to $2x10^8$ mouse LD_{50}/ml. Various microbiological tests and PCR can be used to screen colonies or specimens; virulence can be tested in mice.

Safety Factors

1) Risk of Disease Contraction:

Foodborne botulism occurs after ingestion of foods containing preformed botulinum toxin, most frequently home-prepared and home-preserved foods contaminated with bacteria. Wound botulism occurs when puncture wounds become infected with the organism, which begins to grow and produce toxin, releasing it into the bloodstream. Infant botulism principally occurs in infants under the age of one after ingestion of spores; contaminated honey is suspected to be the cause of most infant botulism. The bacteria colonize the intestinal tract and produce toxin. Bacteria may also colonize the gastrointestinal tract of adults with poor immune systems. Contact of toxin with the skin, eyes, or mucous membranes of the respiratory system can also cause botulism. The intravenous lethal dose of toxin is estimated to be 0.1 to 0.5ng/kg; 0.2 to 1ug/kg if ingested. Person-to-person transmission does not occur.

2) Countermeasures (PPE and medical):

BSL2 containment and procedures should be used whenever materials suspected of containing toxin are handled. BSL3 conditions and biosafety cabinets should be used for any work where aerosols may be generated, or where toxin is produced or purified. PPE should include laboratory coats, gowns, and gloves. Therapy for botulism is still largely limited to supportive care and passive immunization with antitoxin. Supportive care consists of fluid and nutritional support, assisted ventilation (in case of respiratory failure), and the use of antibiotics to combat secondary infections and complications. Antitoxins are available that are effective against all toxin types, but must be administered early. A pentavalent (A to E) toxoid (available from the CDC) can be used for immunization.

Cryptosporidium parvum

Environmental Factors

1) Geographic Distribution:

Worldwide distribution; *C. parvum* oocysts are ubiquitously distributed in the environment. Found globally in drinking and recreational water, and sewage; very common cause of waterborne disease for humans and animals. One study found ~5% environmental water samples contain infectious organisms; *C. parvum* is also a common infection in farm animals. There is often a seasonal variation in incidence of human infection in developed and developing countries, ascribed to waterborne transmission. In tropical climates, infections usually peak during the rainy season. Overall though, there is a higher prevalence in developing countries (infection rate range of 3 to 20%) than developed world (1 to 4.5% infection rate); higher infection rates in AIDS patients (3-20% in US, 50-60% in Africa and Haiti); children and immunocompromised individuals are particularly affected (frequent outbreaks in daycare centers and nursing homes). A 1993 outbreak in Milwaukee infected 400,000 was due to waterborne transmission; but approximately 10% of US infections are foodborne in nature.

2) Disease Symptoms (human and animal):

In humans, principle symptom is profuse watery diarrhea; also often includes dehydration, headache, weight loss, stomach cramps/pain, low-grade fever, nausea, malaise, and vomiting. Symptoms last for 1 to 2 weeks. Some infected individuals are asymptomatic. In mammals, cryptosporidiosis is usually a disease of newborn animals, sometimes causing large outbreaks of diarrhea in calves; calves excrete many more oocytes than adults. Other symptoms include dullness, anorexia and fever; animals usually recover in 2 weeks. Older infected mammals often do not show symptoms, even as they continually shed *C. parvum* oocytes. Serology is often used to confirm outbreaks.

3) Strain information:

Environmental strains exhibit great genetic diversity, but the vast majority are likely capable of infecting humans. Some research shows that isolates can differ greatly in their infectious dose, pathogenicity, and duration of diarrhea, however there is very little information available overall.

4) Reservoirs/Vectors:

Reservoirs are primarily domestic and wild ruminants. A host of small and large mammals (~150 species), poultry, fish, reptiles, and humans are also infected.

5) Agent Sources:

Parasites infect the small intestine, so feces and intestinal biopsies (terminal ileum) harbor *C. parvum*. Lives in Gastrointestinal (GI) tracts of animals and humans; millions of organisms are excreted in stools from beginning of symptoms to several weeks after symptoms resolve. Very often found in diarrhetic feces of calves. Almost all diagnoses are by analysis of stool specimens; feces should be collected during acute stage of illness. Numerous samples should be collected 2 to 3 days apart, as some carriers shed only small numbers of oocysts.

6) Stability:

Thick outer shell enables survival of oocysts for long periods outside body (~2-6 months in moist environment). Oocysts are resistant to low temperatures, high salinity, as well as most disinfectants including 3% hypochlorite, iodophors, 5% formaldehyde, 5% ammonia (prolonged treatment), 10% formol saline, and 3% hydrogen peroxide. One minute of boiling will kill the organism. Oocysts are very resistant to chlorine-based disinfectants.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

Prowazekii Group 1 Agent

2) General Research Volume:

Relatively high volume of laboratory based work (in Pubmed)

3) Survey Information:

No survey information.

4) Former Weapons Activity:

No known former weapons research

5) Culture Collections:

Very common disease in animals and humans; the pathogen is not considered a large biothreat, so it is likely that many diagnostic/clinical and reference labs have samples. It is possible to purchase viable, infectious *C. parvum* oocytes from commercial sources (e.g. Waterborne, Inc, $105/million spores to $1,765/1000 million spores).

6) General Growth Conditions:

Fresh stool is the typical source used for isolating the agent. A large number of commercial diagnostic assays are available for the detection of *Cryptosporidium* oocysts or antigens, but most cannot differentiate between species. Oocysts can also be detected in fecal smears by microscopic analysis (direct immunofluorescence, or more commonly in developing countries, modified acid-fast staining because of low cost, ease of use, and need for basic microscope), but since oocytes of different cryptosporidium species are very similar, PCR is increasingly used to differentiate species. *C. parvum* is an obligate intracellular parasite, so it must be grown in animal tissue culture cells. However, according to the OIE, there is no fully reproducible method for culturing *C. parvum* from body fluids or feces; in vitro cultivation is inefficient because of low parasite yields and oocyst production. More recent development of several media formulations and procedures has been used with some success (MDCK (Madin-Darby Canine Kidney Cells), UltraMDCK, PC-1, UltraCHO (Chinese Hampster Ovaries) and UltraCulture). Once again, microscopy and PCR can be used to detect the organism in culture.

Safety Factors

1) Risk of Disease Contraction:

Fecal-oral route and airborne transmission; relative incidence of infection by each route is not well known. Disease contracted by ingesting contaminated material (water, food). Infectious dose is typically small, around 50 to 100 organisms. Direct human-to-human (especially those with diarrhea) and animal-to-human (with farm animals) contact appears to be common transmission modes. Day care facilities and nursing homes have a high prevalence.

2) Countermeasures (PPE and medical):

BSL2 practices and containment should be used; PPE should include simple latex gloves when handling contaminated material. Good hygiene with frequent and thorough hand washing could significantly prevent infection. Avoid swallowing untreated water, especially in developing countries. Symptoms are typically self-limiting, and there are generally no effective therapeutic agents. Most healthy people will

recover without treatment, but a new drug, nitazoxanide, has been approved for treatment of diarrhea symptoms. No widely accepted vaccines for animals.

Eastern Equine Encephalitis Virus

Environmental Factors

1) Geographic Distribution:

EEEV infections occur primarily in North America; and have been diagnosed in Texas and parts of the eastern United States, mainly along the Atlantic and Gulf coasts, and in southern Canada. Foci are also scattered in the northern portion of South America, and in Central America and the Caribbean. The virus is mostly limited to wet, generally secluded swamps and coastal areas where mosquitoes thrive; disease cases occur in mid-summer to autumn; incidence increases in years of high temperatures and many mosquitoes. In addition, there is sometimes a fluctuation between avirulent and virulent (to people) strains each season; the degree of virulence relates to the host specifics of a given epizootic outbreak. Disease is relatively rare in humans—in US, only 200 cases from 1964 to present; with an average of 4 cases a year.

2) Disease Symptoms (human and animal):

In humans, infection with EEEV often results in no apparent illness. In those who do develop illness, clinical signs range from mild flu-like illness with respiratory symptoms, to meningoencephalitis, stupor, tremors, coma and death. Onset of illness may be very sudden, and duration may be short. The mortality rate is approximately 30%; approximately half of those who survive the disease will have some form of permanent neurological damage. In horses: unexceptionally lethal, causing fever, depression and anorexia before death. In very severe cases, neurological symptoms such as blindness, mental depression and convulsions may occur; obvious external signs (such as lesions) do not exist.

3) Strain information:

EEEV strains are divided into 2 geographic variants or topotyes (North American and South American) based on differences in genomic sequence and antigen expression. The two topotypes differ greatly epidemiologically and in clinical disease expression. North American strains are highly conserved, constituting a unique major lineage, whereas South American strains constitute three major lineages with more antigenic and genetic differences. All the strains in North America and most from the Caribbean belong to the North American lineage, whereas those isolated from Central and South America constitute three South American lineages. The North American strains causes severe disease in humans (mortality 30-80%) and equids (mortality 90-95%). Infection by South American strains can be fatal in equids, but human cases are very rarely seen; serosurveys indicate infection with no clinical disease symptoms.

4) Reservoirs/Vectors:

The virus is maintained in an enzootic transmission cycle between mosquitoes and birds, primarily circulating in mid-summer to autumn. Several species of mosquitoes (and mosquito eggs) act as reservoirs and disease vectors. Infected ticks can also act as vectors, albeit less important ones. Humans and horses are dead-end hosts.

5) Agent Sources:

EEEV infection produces viremia of very short duration. Passerine birds develop high virus titers in their blood, but viremia only last 2-5 days. Viremia in blood of infected horses and humans is also brief, and titers are too small to transfer virus to biting mosquitoes, so animal blood is generally not a viable source for culturing. The virus can be isolated from horse brains (and presumably humans). Tissues should be collected and transported in a viral transport medium (or on a moist sponge).

6) Stability:

EEEV is an enveloped virus that will not survive outside a host. Infectivity inactivated by heat over 56 °C for 10 minutes; virions stable when stored at -40°C to -70°C, not stable at -20 °C or above. Virions are sensitive to treatment with lipid solvents, detergents, ether, trypsin, chloroform, formaldehyde, heat, and ß-propiolactone.

Laboratory Factors

1) Security Classification:

US Select Agent

Prowazekii Group 2 Agent

2) General Research Volume:

Some (low volume) laboratory based work (in Pubmed)

3) Survey Information:

Only 7/722 (0.97%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain EEEV.

4) Former Weapons Activity:

The US had conducted BW research on EEEV.

5) Culture Collections:

Stored in a number of repositories around the world, probably mostly in the US and South America.

6) General Growth Conditions:

Specimens should be prepared (homogenized) in a 10% suspension in phosphate buffered saline (PBS) with bovine serum albumin, penicillin, and streptomycin; then centrifuged to clarify. A number of host systems for virus isolation and amplification have been developed: newborn mice are a sensitive system; intracranial injection will result in infection; brains can later be harvested for virus isolation. Chicken embryos can also be used, although it is a less sensitive system. Yolk-sacs of 6-8 day old embryonated chicken eggs are inoculated, incubated for 7 days, and then the embryo passaged for virus isolation. Virus also isolated in a number of cell culture systems, including Vero cells, rabbit kidney (RK-13) cells, and baby hamster kidney (BHK-21) cells, or in primary cell cultures made of chicken or duck fibroblasts. One ml of suspension is inoculated on a monolayer of cells, allowed 1-2 hours to absorb, maintenance media is added, cultures are incubated for 7 days, and a passage is made. Cytopathic effects are seen starting 1 week later. Recent studies indicate excellent growth of the virus recovered from patient CSF in A549 and MRC-5 cell cultures. Immunohistochemical staining, ELISAs, and RT-PCR can be used to identify virus in clinical specimens (brain tissue or viremic blood) or cultures. Commercial kits are available for detection of agent in specimens and mosquitoes. RT-PCR can be used to test for presence of virus in mosquitoes; hundreds of specimens may possibly be tested in a single day. RT-PCR has also been used for environmental surveillance to detect virus in dead equines.

Safety Factors

1) Risk of Disease Contraction:

Transmitted through the bite of infected arthropods, mostly mosquitoes. No person-to-person (or animal-to-person) transmission.

2) Countermeasures (PPE and medical):

BSL3 containment and practices required for any work with potentially infectious material; ABSL3 required for work with infected animals. PPE such as laboratory coats, gloves, gowns, and eye protection should be worn at all times. In the field, measures that protect against insect bites could help prevent EEE exposure. No effective therapeutic drugs are available; therapy is limited to supportive care currently, with fluid replacement, electrolyte monitoring, etc. Inactivated vaccines are available commercially for equines, but human vaccine is available only for researchers and military personnel. These vaccines are poorly immunogenic and require frequent boosters.

Highly Pathogenic Avian Influenza Virus

Environmental Factors

1) Geographic Distribution:

Highly pathogenic avian influenza, specifically the H5N1 Virus, has circulated in wild birds and poultry primarily throughout Asia, but it continues to expand rapidly worldwide. Nine Asian countries (Korea, Vietnam, Japan, Thailand, Cambodia, Laos, Indonesia, China, and Malaysia) have reported H5N1 outbreaks in birds; the virus is endemic in several of these countries; it is believed eliminated from Japan, Korea, and Malaysia. Bird outbreaks have also occurred in Russia, parts of Central Asia and the Middle East, and Africa. Thus far, HPAI has killed millions of birds; there have been 291 human cases (in Azerbaijan, Cambodia, China, Djibouti, Egypt, Indonesia, Iraq, Laos, Nigeria, Thailand, Turkey, and Vietnam). Indonesia has been particularly hard hit in recent years.

2) Disease Symptoms (human and animal):

Clinical symptoms vary greatly depending on bird species infected, virus strain, host immune system, and environmental conditions. Typical clinical signs are nonspecific; include severe depression, loss of appetite, and vast decline in egg production. On some occasions, sudden death may occur, in the absence of symptoms, with mortality close to 100%. In chickens, symptoms also include dehydration, nasal and oral discharge, respiratory congestion, respiratory tract and gizzard hemorrhages, and diarrhea. Diagnosis difficult, often diagnosed as Newcastle or cholera. Some bird species may shed the virus without any clinical signs (e.g. ducks appear unaffected). In humans, the virus causes severe influenza.

3) Strain information:

Only H5 and H7 avian influenza A subtypes are highly pathogenic to domestic poultry (not all strains, many are lowly pathogenic). A number of H5 and H7 strains can infect humans; H5N1 appears to be particularly virulent and pathogenic. Influenza viruses mutate very quickly; new strains continually arise, prevalence of dominant strains continually changes. Numerous strains have been shown to be resistant to amantadine and rimantadine—drugs often used to treat human illness.

4) Reservoirs/Vectors:

All birds are thought to be susceptible to some extent; the H5N1 Virus has been detected in hundreds of species thus far. Poultry such as chickens and turkeys are particularly susceptible. Migratory waterfowl,

notably wild ducks, appear to be resistant to disease and are considered a significant reservoir. Numerous mammals can be infected, including pets like cats and dogs; mice, ferrets, guinea pigs, macaques, and humans.

5) Agent Sources:

HPAI multiplies quickly in the GI tract of birds; causing systemic infection and severe respiratory symptoms. The virus is present in respiratory fluids and feces (birds may excrete the virus for 10 days or more), and can be collected by taking tracheal, oropharyngeal or cloacal swabs from live (or recently deceased) birds. Cloacal swabs are most likely to yield virus. Organs including the trachea, lungs, air sacs, intestine, spleen, kidney, brain, liver and heart can be processed and may contain adequate virus concentrations. Equipment, vehicles, feed, cages, or clothing contaminated with infected excretions are potential sources. Cage swabs (swabs of fresh fecal matter) may also provide appropriate material; cages housing mixed species are more likely to harbor virus.

6) Stability:

HPAI Viruses can survive for long periods in the environment, especially at colder temperatures.

Laboratory Factors

1) Security Classification:

US Select Agent

Prowazekii Group 3 Agent

2) General Research Volume:

Relatively high volume laboratory/academic research (in Pubmed)

3) Survey Information:

49/722 (6.79%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain HPAI. More than half—26 of the 49—are in Asia (300 labs total).

4) Former Weapons Activity:

No evidence of any BW research.

5) Culture Collections:

Major agricultural and human health concern worldwide; the virus continues to spread rapidly. Thus, many research and clinical/diagnostic laboratories worldwide (particularly in Asia) may be expected to work with live virus.

6) General Growth Conditions:

Specimens (tissues, feces, tracheal and cloacal swabs, etc.) containing virus should be placed in isotonic PBS (pH 7.0–7.4) containing antibiotics (penicillin, streptomycin, gentamycin, mycostatin; higher concentrations for feces samples). Feces and finely minced tissues prepared as 10–20% (w/v) suspensions; all samples kept cold. Conventional method of growing, isolating and characterizing avian viruses requires inoculation of embryonated specific pathogen free (SPF) fowl eggs, or specific antibody negative (SAN) eggs, followed by the infection of chickens with isolated virus. These procedures can be slow, labor intensive and expensive. Feces or tissue homogenate suspensions are clarified by centrifugation, and the supernatants are inoculated into the allantoic sac of at least five 9-11 day old embryonated SPF or SAN fowls eggs. The eggs are then incubated for 4–7 days at 35-37°C; then chilled to 4°C and the allantoic fluids tested for hemagglutination (HA) activity (indicative of Influenza A) or specific H5 subtype (not trivial; generally requires host of antibodies for serology, or RT-PCR). Also, numerous kits have been developed to detect H5N1 in clinical samples, but the specificity and sensitivity are often not well tested. The virus may be concentrated from fluid by ultracentrifugation or precipitation under acidic conditions. Pathogenicity can be determined by sequencing genome segments; but more commonly, a sample is tested by injection in chickens: HPAI if lethal to six or more of the eight 4- to 8-week-old chickens within 10 days following injection with infective allantoic fluid.

Safety Factors

1) Risk of Disease Contraction:

Humans principally infected by close contact with sick birds, typically poultry, contaminated surfaces, or ingestion of undercooked meat. Infection through indirect contact with birds (at wet markets, etc.) may be possible, but unlikely. Human-to-human transmission also appears very unlikely. Virus spreads to birds via contact with contaminated excretions. Increased transmission expected where many birds of numerous species are forced into close proximity, such as at migratory lakes where birds congregate or at wet markets.

2) Countermeasures (PPE and medical):

HPAI should generally be handled in BSL3+ conditions. Clinical specimens can be tested using standard BSL2 practices in a class II biosafety cabinet. Gloves and respirators should be worn at all times. No H5N1 vaccines exist for humans currently, however numerous inactivated, live attenuated, recombinant, and DNA vaccines are in various stages of development. Prophylactic antiviral drugs such as Tamiflu are effective and available for purchase; can typically be used for both prevention and treatment. Some viral strains exhibit resistance to the common drugs amantadine and rimantadine, but oseltamivir and zanamivir appear to remain largely effective.

Mycobacterium tuberculosis

Environmental Factors

1) Geographic Distribution:

Mycobacterium tuberculosis is an aerobic, non-motile and non-spore-forming rod-like bacillus. The *Mycobacterium tuberculosis* complex that causes tuberculosis in humans includes *M. tuberculosis, M. bovis, M. africanum*, and *M. microti.* Infection of *M. tuberculosis* is currently a global pandemic. Estimates report that one third, or 2 billion, of all people are infected. Ninety-five percent of new cases and 98% of deaths occur in developing nations. Susceptible populations include the poor, elderly and overcrowded populations. Prisoners, alcoholics, intravenous drug users and immuno-compromised subpopulations, especially those infected with HIV/AIDs, are at extreme risk. Just 22 developing countries, mostly from Southeast Asia and sub-Saharan Africa, carry 80% of the worlds TB burden.

2) Disease Symptoms (human and animal):

The clinical features of pulmonary tuberculosis include a prolonged cough with thick, often bloody sputum, fatigue, weight loss, night sweats, low-grade fever, dyspnea and chest pain. *M. tuberculosis* can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, bones, joints and skin.

3) Strain information:

There is growing evidence that there are differences between *M. tuberculosis* clinical strains from distinct geographic regions. The Beijing family of *Mycobacterium tuberculosis* strains has been associated with epidemic spread and an increased likelihood of developing drug resistance. This family is widely spread in Asia and has been reported in all continents. There are many strains that appear to be more virulent in the macrophage and animal models such as the common laboratory strains, H37Rv and H37Ra.

4) Reservoirs/Vectors:

Humans

5) Agent Sources:

Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebro-spinal fluid, urine, and in a variety of tissues.

6) Stability:

Mycobacteria spp. are able to survive, without active replication, for weeks to months on inanimate objects if protected from sunlight. They are easily killed by heat (>65° C for at least 30 min) and by UV rays. The organism has a thick, lipid-rich cell wall that renders resistance to harsh treatments (including alkali and detergents) making hospital and laboratory facility decontamination difficult. *M. tuberculosis* is also not affected by freezing or desiccation. *M. tuberculosis* is incapable of replication in the inanimate environment.

Laboratory Factors

1) Security Classification:

Not a US select agent

Prowazekii Group 1 Agent

2) General Research Volume:

Large volume of scientific research (Pubmed)

3) Survey Information:

93/722 (12.88%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *M. tuberculosis*.

4) Former Weapons Activity:

No evidence that *M. tuberculosis* has been used as a bioweapon

5) Culture Collections:

Many laboratories—research and clinical—are expected to have samples.

6) General Growth Conditions:

Compared to other bacteria, the growth of most mycobacterial species is slow, with generation times of up to approximately 20 hours on commonly used media. Most species adapt readily to growth on relatively simple substrates. Optimum incubation temperature is 35-37º C in 5-10% CO_2 or ambient atmosphere. Depending on the species, visible colonies may appear as soon as a few days to up to 6 weeks after incubation under optimum conditions.

Safety Factors

1) Risk of Disease Contraction:

M. tuberculosis is carried by airborne droplet nuclei, 1-5 microns in diameter, when patients with pulmonary tuberculosis cough, sneeze or talk. Particles are suspended in the air infecting susceptible persons via inhalation and establishing infection preferentially in the lungs. Thus, close contact with actively infected people is the most common source of disease contraction. Immuno-compromised people especially those with HIV/AIDS infection and drug users are at extreme risk.

2) Countermeasures (PPE and medical):

Because of the low infective dose (i.e., ID_{50} <10 bacilli) and aerosolization potential of *M. tuberculosis*, clinical and laboratory samples must be considered potentially infectious and handled with appropriate precautions. BSL2 practices and procedures, containment equipment, and facilities are required. All aerosol-generating activities must be conducted in a biosafety cabinet. BSL3 practices, containment equipment, and facilities are required for propagation and manipulation of cultures. Medical countermeasures include first-line antibiotic treatment for several months.

MDR Mycobacterium tuberculosis

Environmental Factors

1) Geographic Distribution:

During the 1990s, Multi Drug Resistant (MDR) *Mycobacterium tuberculosis*, defined as resistance to at least isoniazid and rifampin, emerged as a global threat to TB control. MDR-TB treatment is more extensive, requiring the use of second-line drugs that are costlier and more toxic. While the global burden of MDR *M. tuberculosis* is largely unknown, it most likely present in every country. Regions of the world with the highest burden of MDR-TB include Eastern Europe, Southeast Asia, sub-Saharan Africa and the Western Pacific region. In 2000, three countries, including China, India and the Russian Federation, reportedly accounted for 62% of worldwide cases.

2) Disease Symptoms (human and animal):

Symptoms of MDR-TB are no different from drug-susceptible *Mycobacterium tuberculosis*.

3) Strain information:

Although numerous virulent MDR-TB strains can arise from *M. tuberculosis*, little information exists concerning the genetic characteristics of the specific strains.

4) Reservoirs/Vectors:

Humans

5) Agent Sources:

Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebro-spinal fluid, urine, and in a variety of tissues. *M. tuberculosis* is incapable of replication in the inanimate environment.

6) Stability:

Mycobacteria are able to survive, without active replication, for weeks to months on inanimate objects if protected from sunlight. They are easily killed by heat (>65° C for at least 30 min) and by UV rays. The organism has a thick, lipid-rich cell wall that renders resistance to harsh treatments (including alkali and

detergents) making hospital and laboratory facility decontamination difficult. *M. tuberculosis* is also not affected by freezing or desiccation. *M. tuberculosis* is incapable of replication in the inanimate environment.

Laboratory Factors

1) Security Classification:

MDR-TB is a current NIAID Category C Pathogen but is not a US select agent.

Prowazekii Group 3 Agent

2) General Research Volume:

Moderate volume of scientific research (Pubmed)

3) Survey Information:

93/722 (12.88%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *M. tuberculosis*. Some of these strains are likely MDR.

4) Former Weapons Activity:

No evidence that MDR-TB has been used as a bioweapon.

5) Culture Collections:

Unknown

6) General Growth Conditions:

Compared to other bacteria, the growth of most mycobacterial species is slow, with generation times of up to approximately 20 h on commonly used media. Most species adapt readily to growth on relatively simple substrates. Optimum incubation temperature is 35-37º C in 5-10% CO_2 or ambient atmosphere. Depending on the species, visible colonies may appear after a few days to 6 weeks of incubation under optimum conditions.

Safety Factors

1) Risk of Disease Contraction:

Close contact with actively infected people is the most common source of disease contraction. Immuno-compromised people especially those with HIV/AIDS infection and drug users are at extreme risk.

2) Countermeasures (PPE and medical):

Because of the low infective dose (i.e., ID_{50} <10 bacilli) and aerosolization potential of *M. tuberculosis*, clinical and laboratory samples must be considered potentially infectious and handled with appropriate precautions. BSL2 practices and procedures, containment equipment, and facilities are required. All aerosol-generating activities must be conducted in a biosafety cabinet. BSL3 practices, containment equipment, and facilities are required for propagation and manipulation of cultures. Medical countermeasures include second-line antibiotic treatment for several months.

Rabies Virus

Environmental Factors

1) Geographic Distribution:

Rabies is a common disease globally and is endemic to 5 continents. Developing countries are particularly affected, and Asia and Africa have an especially high disease incidence. There are an estimated 25,000 to 50,000 human cases of rabies a year; only approximately 0.1% of these occur in the Americas and Europe. Asia has, by far, the most cases: in 1997, there were 33,000 Asian cases, 30,000 of these occurred in India alone. In contrast, there were only 32 human cases in the US from 1980 to 2003. Rabies is much more common in wild animal populations; in 2004, approximately 7,000 cases were reported in the U.S; 92% were wildlife cases. Rabies epidemiology is complicated; it differs from continent to continent depending on the fauna and state of developmental progress. Urban rabies occurs mostly in developing countries (in Asia and Africa); stray dogs transmit the disease to people and are reservoirs. The sylvan form is the primary type seen in developed countries; domestic animals come in contact sick wild reservoir animals and then transmit the disease to humans. Island countries may be free of canine rabies but bats act as reservoirs.

2) Disease Symptoms (human and animal):

The rabies virus causes an acute and progressive encephalomyelitis. The prodromal period symptoms are nonspecific and flu-like: nausea, vomiting, headaches, pain/numbness at bite. The acute neurological phase then sets in; most cases present as the encephalitic or furious forms, less than 20% manifest as the paralytic form. Encephalitic symptoms include: restlessness, hyperexcitability, excess saliva, difficulty swallowing, cold sweat, confusion, hallucinations, aggressive behavior, fear of death and water, insomnia, convulsions, spasms, and muscle twitching. Eventually individuals go into coma and cardiac arrest. Paralytic syndromes include: general muscle weakness, head muscle paralysis, bilateral deafness (sometimes), coma, and respiratory and heart failure. In animals, the disease can vary greatly; generally animals are restless, excitable, aggressive, in a daze, and undergo paralysis. Animals may go into hiding or lose fear of people.

3) Strain information:

Rabies strains vary greatly genetically and antigenically; strains are grouped into 7 genetic variants; a panel of 7monoclonal antibodies is commercially available to type the variants. Incubation periods may vary with strains, but all natural viruses appear to be very deadly to human. Strain variants generally circulate within a particular host species in one geographic area.

4) Reservoirs/Vectors:

Nearly all animals are susceptible; the primary reservoirs are wild animals such as raccoons, bats, foxes, jackals, skunks, mongooses, and wolves. Domestic animals and pets such as dogs, cats, cattle, sheep, goats, and pigs can also act as reservoirs. Dogs and cats (pets and strays) are major reservoir species in developing countries, and are key human vectors. In the US, raccoons along the eastern coast are the predominant host species, followed by skunks and foxes; canine rabies has largely been eliminated in developed countries due to effective vaccination programs.

5) Agent Sources:

In infected animals, all tissues and secretions may harbor virus but the highest titers are in the saliva, salivary glands, and the CNS.

6) Stability:

The rabies virus is enveloped and very unstable outside of a host. It is viable for only a few hours in dried secretions or blood under normal environmental conditions, and is rapidly inactivated by sunlight and drying. In cold conditions (winter temperatures; refrigeration), cadavers may harbor live virus from one to several months. The virus is susceptible to heat (50°C for 1 hour), 1% sodium hypochlorite, 2% glutaraldehyde, 70% ethanol, and formaldehyde. One hour acetone treatment does not inactivate rabies virus fully, so special care must be taken during lab work.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

Prowazekii Group 2 Agent

2) General Research Volume:

Relatively large volume of laboratory work (in PubMed); new vaccine work

3) Survey Information:

30/722 (4.16%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain Rabies.

4) Former Weapons Activity:

No known attempts at weaponization.

5) Culture Collections:

Rabies is one of the oldest diseases known to humans; historically very common worldwide particularly in the Developing world. In addition, rabies virus is not often considered a potential BW agent, thus a large number of clinical labs may be expected to have samples.

6) General Growth Conditions:

Rabies virus can be isolated in cell culture or by intracerebral inoculation of mice (classical method) using postmortem CNS tissue—a 20% brain (brain stem and cerebellum) homogenate is best—or from saliva samples taken from living infected animals. Several successful tissue culture systems have been developed; commonly grown in BHK-21 cells, CCL 131 cells, and mouse neuroblastoma cells (available commercially) . Virus can be detected in clinical samples (CNS tissue) and tissue culture by microscopic examination of histopathological stains, a direct fluorescent-antibody test (standard, highly specific, and fast), and immunohistochemical assays, or RT-PCR. RT-PCR is especially useful for the detection of virus in saliva, as well as skin, cornea impressions, tears, eye swabs, and throat swabs—materials on which other tests are not very effective at viral detection.

Safety Factors

1) Risk of Disease Contraction:

Virus is transmitted into open wounds from infected saliva, usually through the bite of an infected animal; virus cannot penetrate intact skin. In the absence of a bite, the virus may penetrate existing scratches, other small lesions, and mucous membranes when infectious material (saliva, contaminated hair or fur, CNS tissue, etc.) comes in contact. Aerosol inhalation is another infection route but rare under natural conditions (documented in bat caves). Infection by non-bite exposure is extremely rare. Human-to-human transmission is possible. Only 1/5 exposed persons develop rabies, it depends on the location of wound and the amount of virus present.

2) Countermeasures (PPE and medical):

BSL2 facilities can be used for diagnostic purposes; BSL3 for any work involving the virus amplification. Minimum PPE should include a lab coat, double gloves, and safety glasses; a biosafety cabinet should be used for handling infectious materials. A face shield and heavy gloves should be worn when dissecting infected carcasses and handling brain matter. In the event of suspected infection, simultaneous postexposure vaccination (active and passive) must be applied within 24 hours after exposure to increase the chance of disease prevention. Bites and scratches should be washed immediately with soap, water, and disinfectants; human rabies serum or immunoglobulin should be inoculated on wounds promptly. If symptoms appear, the person will die. Numerous human and animal vaccines have been developed from whole virions, purified rabies proteins, and recombinant viral vaccines encoding rabies proteins; these are generally quite effective at inducing protective immunity. In the US, a licensed vaccine is administered to individuals (with no prior vaccination) in 5 doses over the course of a month. There are only 3 documented cases of individuals surviving in the absence of vaccination.

Salmonella enterica serotype Typhi

Environmental Factors

1) Geographic Distribution:

Salmonella enterica serotype Typhi (*S. typhi*) is endemic to Central America, South America, Africa, the Middle East, East Asia, and Southeast Asia; several million cases are reported annually. Typhoid fever—the disease caused by this bacterium—is most prominent in developing nations with poor sanitation and increasingly rare in developed countries. Papua New Guinea, Indonesia, and the Philippines have the highest annual incidence of typhoid fever. Typhoid fever is rare in the US (and other developed nations) where approximately 800 cases occur each year; >70% of cases are related to international travel.

2) Disease Symptoms (human and animal):

Typhoid fever is much more severe than the disease syndromes caused by other *Salmonella* serotypes. Symptoms are systemic and typically present as a debilitating high fever and headache. Anorexia, fatigue, chills, stomach pains, and a flat rash (rose spots) in approximately 25% of patients are also possible symptoms. Severe cases may result in hemorrhage, intestinal perforation, blood in feces, rapid rise in pulse rate, hypotension, abdominal tenderness and subsequent rigidity, shock, and altered mental state; extremely severe cases may exhibit delirium. >10% of cases may be fatal. Children usually display mild illness with nonspecific fever. Accurate diagnosis requires lab examination of stool, bone marrow aspirates, or blood.

3) Strain information:

S. typhi is a set of strains grouped according to the immunologic composition of surface antigens; these strains are members of the *S. enterica* species (*S. enterica* subspecies *enterica*). There appear to be two major groups of this serotype: a worldwide set of strains and an African set of strains. Outbreaks seen in South America and parts of Southeast Asia, typically due to the worldwide type, are usually mild (low fatality; low incidence of neurological symptoms); African-type outbreaks in Africa and Indonesia often result in severe illness (high mortality; neurological complications). Great genomic discrepancies, varying virulence, and antibiotic resistance levels are often observed among isolates; MDR resistant strains have appeared in several regions worldwide.

4) Reservoirs/Vectors:

Humans are the sole reservoir for *S. typhi*, carriers may not exhibit symptoms. Insects may act as vectors.

5) Agent Sources:

S. typhi is more frequently isolated from blood cultures than the fecal specimens of infected individuals. 80% of blood cultures taken from typhoid patients during the first week of fever yield bacteria; positive results decrease gradually thereafter. Bacteria may also be isolated from rectal swabs, urine, gall bladder bile, rose spot lesions, or bone marrow aspirates taken from infected individuals. Acquisition of *S. typhi* from a fly vector is unrealistic. The bacteria may also be isolated from contaminated food or water but this is more difficult. Specimens should be placed in a chilled transport medium (buffered glycerol saline or commercial media) and processed quickly.

6) Stability:

The bacteria are resilient and capable of surviving for long periods, perhaps years, in the environment. Virulence is often uncompromised in various conditions. Bacteria are known to be able to survive for 17 days in rabbit carcasses, 62 days in feces, 10-20 minutes on skin, 240 days in ice, up to 30 days in dust, and 130 days in ashes. Survival in sea water is possible up to 9 days; in sewage for weeks. *S. typhi* is sensitive to moist heat (121°C for 15 min); dry heat (160°C for 1 hour); many disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

Prowazekii Group 1 Agent (Prowazekii Group 3 Agent for MDR)

2) General Research Volume:

Relatively high volume of academic research (in Pubmed)

3) Survey Information:

189/722 (26.18%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *S. typhi*.

4) Former Weapons Activity:

Canada and the UK have conducted BW research on *S. typhi*; Japan had weaponized and used *S. typhi* on Chinese military units and civilians. North Korea is believed to have a BW program.

5) Culture Collections:

S. typhi commonly causes outbreaks, some of epidemic proportion, in developing countries. Consequently, it is many research and diagnostic laboratories will likely have specimens.

6) General Growth Conditions:

Numerous protocols are available readily for bacteria isolation. Fecal specimens from acutely ill individuals can often be directly plated; *S. typhi* can also be enriched from fecal samples in Selinite broth before plating. Numerous plating media of varying selectivity can be used for isolation of *Salmonella* (including MacConkey's, eosine methylene blue agar, Salmonella-Shigella agar, xylose lysine desoxycholate agar, Hektoen Enteric Agar, and desoxycholate citrate agar) but the preferred medium for *S. typhi* is bismuth sulfite agar because it is highly selective and can detect lactose-positive *Salmonella* colonies. Most of these media can be purchased readily from commercial sources, but are also easy to make. *S. typhi* can grow in presence or absence of air but is typically grown under aerobic conditions. PCR is usually used to distinguish *S. typhi* from closely related bacteria such as *S. typhimurium*. Commercially available rapid seriodiagnostic tests, biochemical tests and latex agglutination tests can be used to type strains. However, the reliability of these kits varies.

Safety Factors

1) Risk of Disease Contraction:

Human-to-human transmission is by the fecal-oral route; people often contract the disease by ingesting contaminated food or water. Sewage contamination of drinking water (with inadequate chlorination) often leads to outbreaks; poor hygiene is a large contributor to outbreak severity. The infectious dose is typically low—around 10^3 bacteria—and varies according to gastric acidity. Typically communicable through 1st week of illness; 10% of patients discharge bacteria for 3 months after infection; 2-5% are chronic carriers, shedding bacteria for years in absence of symptoms. Although probably extremely rare, flies may transmit bacteria.

2) Countermeasures (PPE and medical):

Any potentially contaminated materials and cultures should be handled in BSL2 facilities. Gloves and lab coats should be worn at all times; thorough hand washing can diminish infection risk significantly. Resistance to one or more drugs (especially ciprofloxacin) is increasingly common; several multidrug resistant strains have been isolated. Chloramphenicol is often used for treatment; however the emergence of resistant strains has led to an increased use of ampicillin, amoxicillin, and trimethoprim; quinolone derivatives like cephalosporins are also used. Several typhoid fever vaccines are in use worldwide (oral and injectable), with efficacy typically ranging from around 60% to 75%; these vaccines typically need a booster every 3 years.

Shigella species

Environmental Factors

1) Geographic Distribution:

Shigellosis is common throughout the world and may be the most common cause of human dysentery. Shigellosis is endemic in developing countries, especially in places with poor sanitation (no running water or plumbing). According to the CDC, "in the developing world, shigellosis is far more common and is present in most communities most of the time." While shigellosis is endemic in tropical and temperate climates, the incidence is higher in warmer regions and during the summer months. Four *Shigella* subgroups cause disease; *S. dysenteriae* 1 (Sd1) commonly causes epidemic dysentery in the developing world but not much in developed countries. In the US (and other developed countries), *S. sonnei* and *S. flexneri* are most common; an estimated 450,000 cases occur each year, but around 20% are related to international travel. Shigellosis is most common at institutions with poor hygienic practices such as child care centers and nursing homes.

2) Disease Symptoms (human and animal):

The symptoms of shigellosis are watery diarrhea (bloody or non-bloody), fever, abdominal cramps, and rectal pain. The symptoms often progress to classic dysentery in which stools contain blood, mucus, and pus. In young children and the elderly (mostly), diarrhea can be so severe that the patient needs to be hospitalized. Severe infection with high fever may lead to seizures (particularly in children <2 years). Some of those infected do not develop noticeable symptoms. All four *Shigella* subgroups can cause dysentery, but Sd1 causes especially severe disease with profuse bloody diarrhea; approximately 5-15% of Sd1 cases are fatal.

3) Strain information:

The *Shigella* genus is divided into four subgroups: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). There are hundreds to thousands of *Shigella* strains, and most do not produce enterotoxin. *S dysenteriae* and *S flexneri* strains are the most virulent generally; *S. dysenteriae* serotype 1 is most virulent of 4 subgroups; is the only cause of epidemic dysentery; and the strains are often capable of releasing Shiga toxin. *S. sonnei* strains often cause asymptomatic infection.

4) Reservoirs/Vectors:

The only natural reservoirs of *Shigella* are humans and other large primates; humans are the primary reservoir. Humans shed bacteria in large numbers during the acute stage of disease when they are sick, and for 1-4 weeks afterwards. Flies may act as vectors.

5) Agent Sources:

Shigella bacteria are mostly isolated from the intestines of infected humans; dysentery can be caused by numerous organisms, so *shigella* species must first be detected in bloody stools before isolation. Positive cultures are most often obtained from blood-tinged plugs of mucus in freshly passed stool specimens obtained during the acute phase of disease. Whole stools and rectal swabs may also be used to culture *Shigella*. The specimen should be transported and processed rapidly or deposited in a chilled buffered glycerol saline holding solution (or commercial transport media); it should be frozen after 3 days. Bacteria may also be isolated from food or water contaminated with feces, but the organism probably does not live freely in the environment.

6) Stability:

The bacteria may survive in feces for 11 days, flies for up to 12 days, water for 2-3 days, and on clothing for 8 days. *Shigella* bacteria are sensitive to moist heat (121°C for 15 min) and dry heat (160°C for 60 min). Disinfectants such as 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde kill the bacterium.

Laboratory Factors

1) Security Classification:

Not a US Select Agent

Prowazekii Group 1 Agent

2) General Research Volume:

Relatively high volume of academic research (in Pubmed)

3) Survey Information:

110/722 (15.24%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *S. dysenteriae*.

4) Former Weapons Activity:

Japan had weaponized *Shigella*, Canada conducted BW research on *Shigella*.

5) Culture Collections:

This organism is well characterized, frequently causes outbreaks throughout world, and is not a US Select Agent. Consequently, a large number of culture collections and diagnostic/clinical laboratories very likely have numerous samples and strains.

6) General Growth Conditions:

Scientists and clinicians have been growing *Shigella* for a long time; many effective methods have been developed. A variety of basic, easy to make media can be used to grow bacteria; most of this media can be purchased pre-made commercially. *Shigella* isolation typically involves initial streaking of sample on a more selective media with aerobic incubation to inhibit the growth of the anaerobic normal flora, followed by streaking on a less selective media such as MacConkey. An enrichment step is sometime used beforehand [growth in "*Shigella* broth" (very basic; recipe online) with novobiocin] to select against other bacteria present in the sample, however no enrichment media is suitable for all isolates. A liquid enrichment medium (Hajna Gram-negative broth) may also be inoculated with the stool specimen and subcultured onto the selective/differential agarose media after a short growth period. Commonly used primary isolation media include xylose lysine desoxycholate agar, Hektoen Enteric Agar, desoxycholate citrate agar, and Salmonella-Shigella agar (SS). SS media may inhibit the growth of some Sd1 strains. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (coliforms) from non-lactose fermenters such as *Shigella*. Following overnight incubation of primary isolation media at 37° C, colonies are very small, slightly pink and translucent, and non-lactose-fermenting; colonies can be further evaluated using PCR and/or a variety of other selective media. *Shigella* bacteria can be difficult to distinguish from *Escherichia coli* antigenically. Plasmid profiling and restriction fragment length polymorphism (RFLP) analysis used for subtyping.

Safety Factors

1) Risk of Disease Contraction:

Human-to-human transmission via direct or indirect fecal-oral route; as few as 10-200 bacteria may be enough to cause infection; communicable particularly during acute stage of illness. Ingestion of contaminated food can lead to infection; can also be acquired by drinking or swimming in contaminated

water. Water may become contaminated if sewage runs into it or if someone with shigellosis swims in it. Children are particularly at risk: 2/3 of all cases are children.

2) Countermeasures (PPE and medical):

Contaminated material should be handled in BSL2 facilities. Gloves should be worn whenever infected materials are handled. Spread of *Shigella* is best prevented by frequent and careful handwashing with soap. The illness usually resolves in 5-7 days in the absence of any treatment; severe cases can be treated with antibiotics such as ampicillin, trimethoprim/sulfamethoxazole, nalidixic acid, or ciprofloxacin. However, antimicrobial resistant strains (many MDR strains in developing world) are thought to be common. Antidiarrheal agents should not be used. No vaccine exists to prevent shigellosis.

Yersinia pestis

Environmental Factors

1) Geographic Distribution:

Y. pestis is endemic in large numbers of small natural foci (corresponding to rodent populations) present in Africa, the FSU, the Americas, Asia and Middle East; foci cover approximately 6-7% of global land area. There are consistently only ~2 to 3,000 human cases per year (probably an underestimate); highest incidence is in Africa (accounts for over 90% of cases worldwide). Most active African foci in eastern and southern regions (Ituri province in DRC is most affected area); Andean mountain region and parts of Brazil contain most active S. American foci. 90% of US cases occur in New Mexico, Arizona, Colorado, and California.

2) Disease Symptoms (human and animal):

Three forms of plague: A) Bubonic plague most common; nonspecific early symptoms (flu-like: chills, headache, fatigue); very obvious later in illness (lymph gland swelling and formation of painful buboes). B) Septicemic plague in small percent of cases; symptoms similar to bubonic form minus the buboes; meningitis and pneumonic disease more likely. In severe cases, necrosis of acral regions (nose, digits) may occur; mortality very high. C) Pneumonic plague is rare and resembles an array of pneumonia-like respiratory illnesses (high fever, cough, chest pain). Plague diagnosis can be difficult early on; gram-negative staining in sputum or bubo aspirates can be used for presumptive diagnosis.

3) Strain information:

Y. pestis species are divided into 3 biovars (Antiqua, Medievalis, Orientalis) according to genomic data and ability to ferment glycerol and reduce nitrate. The number of strains is estimated to be very large; strains vary greatly (genotypically, in virulence, etc.) due to the large number of distinct, isolated foci and large numbers of host/vector species; variation in plasmid content also affects virulence and drug resistance. Plasmid variation appears to be larger in Asian strains. Strains of the Orientalis biovar cause most modern plague.

4) Reservoirs/Vectors:

Wild rodents (over 200 species) are the natural reservoir; wild rodent fleas (over 80 species), especially the oriental rat flea, are vectors. Rodents exhibit inapparent infection; fleas may be infective for months. Also circulates among animals such as rabbits/hares and various carnivores. Humans occasionally contract bacteria. Large rodent die-offs frequently prelude human outbreaks

5) Agent Sources:

Bubo aspirates and blood are most appropriate for culture. Sputum, throat swabs, urine, feces, animal carcasses and infected tissues are also potential sources; infected fleas may also yield bacteria. Bubonic plague patients shed bacteria intermittently, so numerous specimens should be collected in 24 hour period; specimens should be collected before antibiotic treatment.

6) Stability:

Bacteria can survive for weeks outside of a host; viable in blood for 100 days; in dried blood for 3 weeks; in flea feces for 5 weeks. The bacteria may remain in infected human bodies for up to 270 days; and can survive in soil for extended periods. Bacteria are killed after 15 minutes at 55°C, and by exposure to sunlight for 3-4 hours. Susceptible to most disinfectants including 1% sodium hypochlorite, 70% ethanol, 2% glutaraldehyde, and formaldehyde.

Laboratory Factors

1) Security Classification:

US Select Agent

Prowazekii Group 3 Agent

2) General Research Volume:

Relatively large volume of research (in Pubmed); attracting more research because potential BW agent. Labs throughout the world conduct research.

3) Survey Information:

36/722 (4.99%) labs surveyed in Asia, the Middle East, Latin America and Eastern Europe contain *Y. pestis*.

4) Former Weapons Activity:

Canada, the UK, the US, Germany, South Africa, and Iraq (perhaps) conducted BW research on *Y. pestis*. The Soviet Union had weaponized the bacteria, producing large quantities for incorporation into aerosol weapons. Alibek estimates that more that 10 institutes and thousands of researchers worked with plague. North Korea likely has an active *Y. pestis* BW program.

5) Culture Collections:

Present in numerous legitimate culture collections in West under high security; it is a good assumption that samples are present in smaller, unregistered collections overseas. North Korea had likely attained *Y. pestis* from a Japanese culture collection.

6) General Growth Conditions:

Many bacteria species grow faster than *Y. pestis*, so isolation from sputum or carcasses can be difficult; also not easily culturable from postmortem tissue samples. Buboes should be thoroughly flushed with saline to maximize isolation chances; several blood cultures should be collected. If mixed flora contaminants or little *Y. pestis* bacteria, passage through mice may increase isolation chances (infected fleas have been pooled and used to infect mice). Numerous culturing protocols available: can grow well on conventional nutrient rich media at 28°C in 5% CO_2 but selective media may be required for environmental samples. WHO and CDC recommend sheep blood agar, brain heart infusion agar, MacConkey agar, eosin methylene blue agar for relatively sterile samples such as blood or lymph node aspirate. There is also a variety of more selective media developed for clinical/environmental samples, including brain heart infusions with Irgasan, cholate salt, crystal violet, and nystatin. Typically 48 hours for observable growth (1-2 mm in diameter colonies); on sheep blood plates, colonies are gray to yellowish, opaque, and exhibit an irregular "fried egg" appearance; on MacConkey small, nonlactose fermenting colonies. No hemolysis on blood agar media. Difficult to predict virulence of strains in humans; guinea pig virulence and rhamnose fermentation appear to be best indicators. Stains with Wright, Giemsa or Wayson's dye; fluorescence microscopy (antibody staining of F1 antigen), and antigen capture ELISA, and PCR are more specific and often used to identify the organism. Commercial systems are generally inaccurate in identification of *Y. pestis*.

Safety Factors

1) Risk of Disease Contraction:

Y. pestis is transmitted principally by flea bites and much less often by contact with (bites, scratches) infected animals. Primary septicemic plague may develop after a flea bite, or may arise secondarily from bubonic disease. Direct person-to-person transmission of bubonic and septicemic plague is not seen. Bubonic and septicemic plague may progress to pneumonic plague if bacteria spread to lungs (very

rare); or if aerosol droplets or contaminated material is inhaled directly; person-to-person spread of pneumonic plague is very rare. Transmission may occur from 2 meters away; overcrowding facilitates spread of pneumonic plague. Vast majority of human plague cases occur from flea bites; handling of infected carcasses can also be dangerous.

2) Countermeasures (PPE and medical):

BSL2 facilities, practices, and equipment should be used to handle any clinical material or cultures potentially containing *Y. pestis*; BSL3 when aerosols may be generated (centrifugation, milling, animal use, etc.). Respirators are effective at preventing transmission if aerosols are present; gloves, gowns and eye protection should be worn if infected material or animals are handled. Host of effective antibiotics—most strains susceptible to streptomycin, gentamycin, doxycycline, chloramphenicol, ciprofloxacin, and tetracycline—although must be administered early at development of fever/cough. Pneumonic plague is almost 100% fatal if not treated within first 24hours of symptoms. Drug resistant strains are rare, a few isolates have been resistant to streptomycin or tetracycline; only one isolate from Madagascar has been multidrug resistant. Vaccines are sometimes used for laboratory personnel, but are not widely available; a booster is often required every 6 months. Tetracyclines are typically used for prophylaxis.