BIOTERRORISM AND THE CLINICAL LABORATORIAN

The Role of the CLS - Microbiologist

INTRODUCTION

The events that took place in the United States in Fall of 2001 had a profound effect on the entire country and the world. The terrorist attack on the World Trade Center, and the subsequent anthrax bioterrorism attack, brought to the forefront the world's vulnerability to massive acts of terror. The clinical laboratory scientist working in the microbiology laboratory played an extremely important role in the anthrax attack through isolation and identification of the pathogen. This attack brought to light the need to educate the public and health care professionals about agents of biological and chemical warfare.

The role of the clinical laboratory scientist working in the microbiology laboratory is key in suspected outbreaks of a bioterrorism event. Until the microbiologist identifies the pathogen, other health care providers don't know if they are dealing with the agent. They may suspect, and have good clinical clues to suspect the agent, but definitive identification comes from the microbiologist. Thus, the clinical microbiologist is the gate keeper, the final word, and plays a key role as a first responder in suspected bioterrorism events. The role of the laboratorian is multi-faceted. Clinical laboratory professionals must know:

- Your lab's position within the Laboratory Response Network
- Procedures and protocols
- Safety precautions
- When to refer isolates to a higher level lab
- When to set up work in isolation
- Where the next level labs are located in the geographical area
- How to appropriately package samples for transport to another lab

COURSE GOALS

The goal of this activity is to educate clinical laboratory professionals working in Sentinel Laboratories of the Laboratory Response Network (LRN) about their role as first responders in a bioterrorism event or suspected BT event.

OBJECTIVES

- Discuss the historical perspective of biological and chemical warfare agents
- Define CBRNE
- List examples of chemical warfare agents (CWAs)
- Describe clinical symptoms of CWAs
- Explain the effect of CWA's on laboratory data/tests findings

- Briefly discuss radiological, nuclear and explosive devices as agents of warfare
- Describe key components of a Laboratory Preparedness
- Define Laboratory Response Network (LRN)
- Explain the responsibilities of the three levels of LRN labs
- List the CDCs top agents of biological warfare designated as Category A, Category B and Category C agents
- Discuss the pathogenesis and clinical features of Category A agents
- Discuss the pathogenesis and clinical features of select Category B agents
- Describe specimen selection, collection, transport, safety, reporting, and specimen disposal protocols for select agents
- List the biochemical tests and reactions used by a sentinel lab to rule out Category A and select Category B agents
- Compare and contrast the colonial morphology, microscopic morphology, and biochemical profile of select agents with other similar bacteria

HISTORICAL OVERVIEW

The use of terror tactics, both biological and chemical is not an invention of modern times. Terrorist events have been recorded in history as early as 423 B.C., when terrorist tactics where used in the Peloponnesian War. History records terrorist acts in 7th Century A.D. Greece, 15th and 16th Century Venice, the Napoleonic Wars, up to present times. Some of the ancient terror acts included poisoning the enemy's well water with purgative herbs, and hurling the dead corpses of smallpox victims over the walls of the enemy fortress. More recent threats included:

- 1984 Rajneeshee cult incidents of contaminating salad bars with Salmonella in Texas
- 1995 Aum Shinrikyo releases Sarin in Tokyo subway; found with an arsenal of biological weapons

For a comprehensive chronological overview of the history of bioterrorism click on: **History of Bioterrorism**

TYPES OF TERRORIST ATTACKS

Terrorist attacks are categorized as overt or covert attacks.

Overt attack:

- Announcement
- Immediate impact
- First responders: police, firefighters, EMS personnel

Covert attack:

- No immediate request/announcement
- Targets are hit

- First responders: physicians, primary care providers, private and public health care system
- Causalities of a covert attack will present first to:
  - Private physicians
  - Emergency rooms
  - Hospitals
  - EMO's
  - Outpatient clinics

Who are terrorists?

- Lone individuals
- Identified local or non-aligned terrorist groups
- Internationally sponsored groups
- Doomsday cults
- Insurgents

CBRNE (pronounced - "see-burn-ee")

Chemical agents of terror/warfare

Biological agents of terror/warfare

Radiological agents of terror/warfare

Nuclear devices

Explosive devices

Terrorists use CBRNE agents because:

- Agents are available and relatively easy to manufacture
- Large amounts are not needed in enclosed spaces
- CBRNE incident is difficult to recognize
- Easily spread over a large area
- Psychological impact - fear factor
- Can overwhelm existing resources - impact on commerce, economy, society

Though the primary focus of this learning activity will be on the agents of biological warfare, it is essential that clinical laboratory professionals understand the scope of all agents of terrorism/warfare: chemical, biological, radiological, nuclear and explosive. A brief overview of chemical, radiological, nuclear and explosive agents will be presented, followed by an in depth review of biological agents.

CHEMICAL WARFARE AGENTS

A chemical warfare agent (CWA) is any chemical used for military purposes, which may cause temporary loss of performance, permanent injury or death. There are thousands of poisonous substances, and about 70 have been used and/or stockpiled as chemical weapons in the 20th-21st century. Presently, only a few are of interest:

- Nerve agents
- Vesicants
- Cyanide
- Riot control agents
- Psychotomimetic agents

The key characteristics required of a chemical warfare agent are:

- Toxic, but not too difficult to handle
- Can be stored for long periods of time
  - Does not destroy or corrode packaging material
- Retains effectiveness when dispersed
  - Relatively resistant to atmospheric H_2O and O_2
- Withstands heat of dispersal

How do they work?

- Causes biological effects by inhibiting acetyl cholinesterase (AChE)
- Acetylcholine (ACh) accumulates
- Clinical effects are due to excess acetylcholine

Nerve Agents:

- All organophosphorous compounds
- Affect the nervous system transmission
- Designed to produce an acute lethality
- Manufactured from inexpensive and readily available materials
- Fairly simple techniques used in production
- Stable liquids, usually clear, colorless; easy to disperse
- Tasteless, most are odorless
- Penetrates skin and clothing
- Examples:
  - GA (Tabun)
  - GB (Sarin) - mainly inhaled
  - GD (Soman)
  - GF - skin uptake
  - VX - persistent in environment, skin uptake
  - Volatility: GB>GD>GA>GF>VX
- Symptoms:
  - Low dose exposure:
    - Salivation, miosis, headache, nausea
  - Intermediate dose exposure:
    - Bronchodilation, mucous secretions, GI cramping, nausea, muscle weakness
  - High dose exposure:
    - Convulsions, loss of consciousness, muscle paralysis

- Death due to asphyxia

Vesicants:

- Produce vesicles or blisters
- First used in battlefields in WW I (Mustard sulfide)
- Documented use of mustard agent in Iran-Iraq War 1982-1988
- Simple to manufacture
- Onion, garlic, mustard like smell
- Pale yellow to dark brown in color
- Oily liquid
- Persistent in environment; hugs the ground
- Causes loss of performance rather than acute lethality
- Usually requires a lengthy hospitalization
- Damage cells and tissue by direct damage to DNA by cross linking
- Effect is dose dependent
- Symptoms:
  - Effects eyes first (tearing, inflammation, pain and corneal damage)
  - Skin & lung effects in 2-24 hours
  - Leukopenia seen 3-4 days following exposure
  - Increased risk of infection
  - Cancer in the long term

Cyanide:

- One of the least potent agents
- Hydrogen cyanide gas
  - Highly volatile
  - Lighter than air
  - Difficult to achieve high concentrations in large area
- Cyanide chloride gas
  - Less volatile, heavier
- Rapid lethality with inhalation of high dose (confined area or near site of release)
- Death by histotoxic hypoxia
  - Inhibits cytochrome oxidase
  - Stops aerobic cell metabolism

Riot Control Agents:

- Tear gases
  - Aerosols of solid materials
- Achieve temporary incapacitation
  - Tearing, eyelid cramping, pain, respiratory irritation, burning of mucous membranes
  - Lasts <30 minutes once "fresh air" is reached
- Decontaminate skin and clothing
  - Use dilute alkaline solutions

Psychotomimetic Agents:

- Incapacitate by causing psychological or behavioral disorders at low doses
- Possible agent categories:
  - Stimulants
    - Example: Amphetamine
    - Usefulness: insufficient potency, counterproductive
  - Depressants
    - Example: Haloperidol
    - Usefulness: insufficient potency, little sedation
  - Psychedelics
    - Example: LSD
    - Usefulness: unpredictable behaviors
  - Deleriants
    - Example: Anticholinergics
    - Usefulness: most potential
      - Vision impairment
      - Hyperthermia
      - Dry mouth
      - "Walking dream" state
      - "Phantom" behaviors
      - Paranoid tendencies

Potential Chemical Weapons:

Other potential chemical weapons can be biotechnology-developed molecules that are based on the structure and activity of existing substances. Such biotech-engineered molecules could target organs. Biotechnology techniques could potentially be used for large-scale production of naturally occurring toxins.

For more detailed information review this site:
Chemical Agents of Warfare

RADIOLOGICAL THREAT

There is a growing concern of the use of Radiological Dispersal Devices or "Dirty Bombs". This type of warfare was seen in 1995 with the Chechnya attack on Moscow Park. Some examples of agents of radiological warfare:

Americium-241
Cesium-137
Cobalt-60
Iodine-131
Other iodine radioisotopes (I-132, I-134, I-135)

Phosphorus-32
Plutonium-238
Plutonium-239
Polonium-210
Radium-226
Strontium-90
Technetium-99m
Thorium-232
Tritium
Uranium-233
Uranium-238
Zirconium-95

Radiation:

- Affects dividing cells by damaging the DNA, preventing replication
- Sublethal dose (1-3Gy)
- Immediate increase in peripheral blood neutrophils
- Thrombocytes decrease in 3-4 days - increased bleeding time
- Affects on granulocytes and lymphocytes - increased risk of infection

If you are interested in more detailed information, view the following sites:

Radiation and Health Effects

Dirty Bombs

NUCLEAR THREAT

As with a radiological threat, there is an increasing concern of the potential of a nuclear attack. The world is aware of many countries that have nuclear weapons, and also of the potential of countries that have not openly been known to have nuclear weapons, to posses them. The question of moral, ethical and general world safety issues must be considered. It is the hope that international agencies such as the United Nations, NATO and other cooperative international groups to diplomatically monitor and develop appropriate protocol that safeguard the entire world. However, the threat may still be present, and the world must be on alert. For more detailed information on nuclear terrorism view the following site:

Nuclear Terrorism & Health Effects

EXPLOSIVE DEVICES

Explosive devices can come in big and small packages. On a smaller scale, a hand held home-made bomb can do much damage in a small area such as a shopping mall. On a large scale, there are historical events such as the high explosives used to destroy the USS COLE and of course, the use of an aircraft as an explosive device in the attack on the World Trade Center on September 11, 2001.

For a more detailed overview, the following site lists key points of chemical, radiological and nuclear agents:
Chemical, Radiological & Nuclear Warfare Fundamentals

This brief overview demonstrates that chemical, radiologic, and nuclear agents have a primary effect on many organ systems including the respiratory system (chemical agents), the central nervous system (nerve agents, toxins), and the integumetary system (vesicants). The clinical effects of CWA's can include pulmonary edema, excessive production of watery secretions, respiratory distress, dyspnea and even complete airway obstruction. CBR agents can damage cells and tissue by direct damage to the DNA by cross-linking. The chemical and hematopoiesis parameters analyzed in the clinical laboratory will be effected. Clinical laboratory scientists should be aware of the laboratory findings following exposure to chemical, radiological and nuclear agents of warfare and how the laboratorian plays a part in the diagnosis of these conditions.

BIOLOGICAL AGENTS OF WARFARE

The laboratorian plays a key role in identifying biological agents and preventing their spread. The laboratorian is one of many healthcare providers that serve as a first line responder in a potential bioterrorism attack. The CDC guidelines emphasize that clinical lab personnel will most likely be the first ones to perform preliminary testing on clinical specimens from patients who may have been intentionally exposed to organisms, and that they will play a critical role in facilitating rapid identification of these agents of bioterrorism. Health institutions and the laboratory must have safety and security procedures in place. Specific protocols are needed to ensure the protection of laboratory personnel, and to follow chain of custody requirements.

THE INSTITUTION PLAN:

Every institution should have a response plan that is institution specific. The plan begins with parameters that outline the process of activation of a large comprehensive emergency management plan/network within the institution as well as the protocols to notify local emergency response teams and transfer to acute care hospitals.

Elements of the Plan:

- **Operational Planning includes-**
  - Chain of command in the institution
  - Notification, internal and external
  - Authority Statement
  - Education of ED staff
  - Hospital electronic media
  - Local news agencies

  - Healthcare worker education

- **Reporting and Contacts -**

Internal	External
Infection Control	Local Health Dept.
Epidemiologist	FBI
Administration	State Health Dept.
Public Affairs	CDC
Legal Dept.	Ministry of Health

- **Inventory Preparedness-**
  - Hospital and long-term care beds in system or region
  - Isolation capabilities
  - Decontamination units
  - Emergency medical services
  - Therapeutic modalities
    - Antimicrobial stockpiles
    - Ventilators
    - Antisera
    - Vaccines

- **Components of Facility BT Readiness Plan-**
  - Patient management protocols
    - Isolation precautions
    - Patient placement
    - Patient transport
    - Cleaning & disinfection
    - Discharge management
    - Post-mortem care
  - Post-exposure management protocols
    - Decontamination
    - Prophylaxis/post-exposure immunization
    - Triage & management of "large scale" exposures
    - Psychological aspects
  - Public information readiness
  - Laboratory readiness
    - Observing safety precautions
    - Obtaining diagnostic samples
      - Critical to optimum recovery
    - Laboratory processing criteria
      - Know what to do
      - Know what to look for
      - Know when to stop
      - Know what to send out to appropriate reference lab
      - Know where to send it
      - Know how to send it
    - Transport requirements

Additional readings:

Bioterrorism Readiness Plan: A Template for Healthcare Facilities

The Expanding Role of Microbiology

BIOSAFETY LEVEL GUIDELINES

The CDC has established biosafety level guidelines to ensure the safety of professionals working in microbiological and biomedical laboratories. The guidelines provide recommendations for safety practices and techniques, necessary safety equipment and required physical facilities. Most hospitals and independent laboratories should utilize BSL-1 through BSL-3 safety practices.

BSL-1:

- Standard microbiological practices
- Primary barriers/equipment:
  - No special safety equipment required
- Facilities: Open bench-top sink required

BSL-2:

- Agents associated with human disease, usually not aerosol
- BSL-1 practices plus:
- Limited access
- Biohazard warning signs
- "Sharps" precautions
- Biosafety manual defining any needed waste decontamination or medical surveillance policies
- Primary barriers/equipment:
  - Class I or II BSC
  - PPEs: protective lab clothing, gloves, respiratory protection as needed
- Facilities: BSL-1 plus
  - Autoclave
  - Eyewash

BSL-3:

- Agents with serious/lethal consequences, aerosol potential
- BSL-2 practices plus:
- Controlled access
- Decontamination of all waste
- Decontamination of lab clothing before laundering
- Baseline serum
- Primary barriers/equipment:
  - Class I or II BSC

  - PPEs: protective lab clothing, gloves, respiratory protection as needed
- Facilities:
  - BSL-2 plus:
  - Physical separation from access corridors
  - Self-closing, double door access
  - Exhausted air not re-circulated
  - Negative airflow into lab

BSL-4:

- Dangerous, exotic agents which pose high risk of transmission to lab personnel or related agents with unknown transmission
  - Example: Smallpox, Viral Hemorrhagic Fevers
- BSL-3 plus:
- Clothing changes before entering lab area
- Shower on exit
- All materials decontaminated on exit from facility
- Primary barriers/equipment:
  - All procedures conducted in Class III BSC, or in Class I or II BSC in combination with full body, air-supplied, positive pressure personnel suit
- Facilities:
  - BSL-3 plus:
  - Separate building or isolation zone
  - Dedicated supply and exhaust, vacuum, and decon system
  - Other requirements as outlined by CDC

Additional readings:
CDC Biosafety Criteria

1,2,3's of Biosafety Levels

Laboratory Response Network

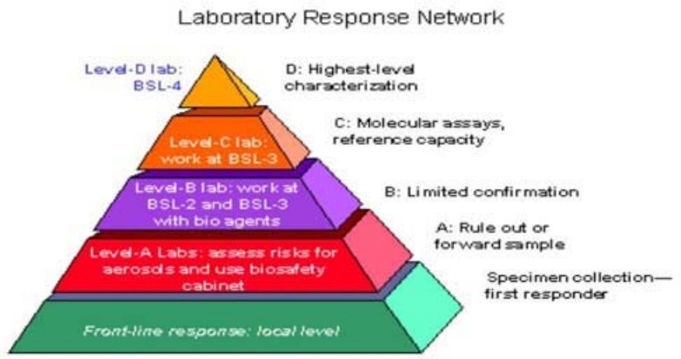
What is LRN & why was it established?

Presidential Decision Directive 39, issued in 1995, outlined national anti-terrorism policies and assigned specific goals to federal departments and agencies. In response to this directive, the FBI and the Association of Public Health Laboratories collaborated to establish the Laboratory Response Network (LRN) in 1999. The objective was to ensure an effective laboratory response to bioterrorism by helping to improve the nation's public health infrastructure.
The purpose of the LRN is to maintain an integrated network of laboratories both nationally and internationally, that can respond to bioterrorism and chemical terrorism. Comprehensive and standardized diagnostic and analytical testing are to be provided by this unique, interconnected system of laboratories. The key word is "standardized". One of the goals is to assure that all laboratories are following the same protocol for isolation

and identification.

How has the LRN evolved?

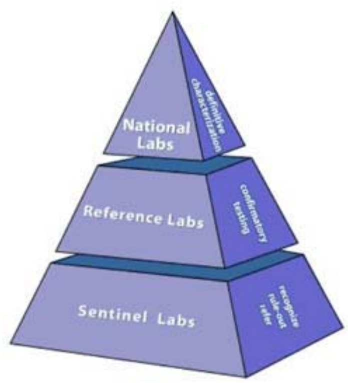
When first established, the LRN was divided into four levels:



http://www.homelandsecurity.org/journal/images/niemyer_fig.gif

- **Level A Lab- (clinical labs)**
  - Detect early (presumptive cases)
  - Adequate safety to rule out critical biological agents
  - Must have certified biological safety cabinet (BSL -2)
  - **RULE OUT AND REFER**
- **Level B Lab- (public health labs)**
  - Level A lab capacity plus:
  - BSL-3 safety facility (recommended)
  - Antimicrobial susceptibility testing
  - Isolate & identify
  - **RULE IN & REFER**
- **Level C Lab- (typing labs, public health labs)**
  - Level B lab capacity plus:
  - BSL-3 safety facility
  - Rapid identification
  - **RULE IN & REFER**
- **Level D Lab- (CDC)**
  - Level C lab capacity plus:
  - BSL-4
  - High level characterization (molecular)
  - Secure banking of isolates
  - Probe for universe of agents

Since 1999, the LRN has evolved to a three tier format, and expanded partnership beyond clinical diagnostic laboratories.



http://www.bt.cdc.gov/lrn/biological.asp

The revised three-tier system:

- **Sentinel Laboratory (Level A labs)**
  - BSL-2
  - Members: hospital-based labs, clinical institutions, commercial diagnostic labs
  - Voluntary participation
  - Perform microbiology
  - Capable of following Sentinel Protocol
  - Early detection of agents via a “rule out” protocol
  - Refer suspect agents to LRN Reference Lab using appropriate protocol
- **LRN Reference Laboratory (Level B & C labs)**
  - Members:
    - State Public Health Labs
    - Large city or county labs
    - Military labs
    - Veterinary labs
    - Agricultural labs
    - Food & Water labs
    - International labs
  - BSL-3
  - Registered with the LRN
    - Connected through a secured network
- **LRN National Reference Laboratory (Level D lab)**
  - Highest biosafety level
    - BSL - 4
    - Can work with highly infectious agents (smallpox, Ebola, etc.)
  - High level of characterization (molecular)

Additional reading:

The Laboratory Response Network for Bioterrorism

ROLE OF THE SENTINEL LABORATORY

The role of the Clinical laboratory Scientists working in the Sentinel Laboratory is:

- **Be alert and aware of the possibility of bioterrorism agents**
- **Be able to "rule out" threat agents**
- **Be able to recognize cellular and colonial morphological characteristics of these agents**
- **Be able to follow the institutions and state's protocol to handle potential threat agents**

Institution and Local/State Health Departments BT Preparedness

Local preparedness is essential. The initial detection of these agents will most likely occur locally, thus surveillance is needed at the city and state level. The initial response will also be local. The city/county/state officials need the sources and protocols to deal with the potential bioterrorism attack. Partnerships are needed between institutions, local and state departments of health, the CDC, federal agencies and world health agencies such as WHO (World Health Organization).

Clinical Laboratory Scientists should be familiar with their institution's plan. Find out about your institutions plan. Who is on the team? What part will you play as a clinical laboratorian? Is a laboratorian on the planning committee? This is a vital component of an institution's preparedness, and clinical laboratory scientists should play a part on this institutional administrative team effort. In addition to the institution plan, laboratorians need to know the protocol established in their microbiology department to handle suspect agents. Key topics laboratorians should be familiar with are:

- Select Agent Program (SAP) - federal program that governs the possession, use and /or transfer of agents or toxins deemed a threat to the public, animal or plant health, or to animal or plant products
    - Examples of *some* agents/toxins included in the list:
        - *Bacillus anthracis*
        - *Yersinia pestis*
        - *Francisella tularensis*
        - *Brucella abortus; Brucella melitensis; Brucella suis*
        - *Burkholderia mallei; Burkholderia pseudomallei*
        - Variola virus
        - Viral hemorrhagic fevers
        - Botulinum neurotoxins
    - Institutions must be SAP approved to work with select agents/toxins

In the event a select agent has potentially been "ruled in" as suspect by laboratorians working in a Sentinel lab, the agent must be:

- Transferred to an SAP approved facility (usually a state health lab)
- Remaining cultures and original specimen materials must be destroyed

- Check institution and state protocols for reporting, handling/transport, destruction and documentation
  - Each state will have specific protocol and some type of clinical submission forms for the clinical laboratory scientists at Sentinel labs to complete when sending specimens to the next level in the LRN chain (Reference Labs)
- Check CDC requirements for documentation (CDC Form 0.1318)
  - Report of the Identification of a Select Agent or Toxin in a Clinical or Diagnostic Laboratory
    - This form must be completed by the laboratorian at the Sentinel Lab

- Packaging and Shipping Guidelines
  - Specific standards established to meet regulatory requirements of the United States Postal Service as per the International Air Transport Association (IATA).
  - Certified individuals at the Sentinel Laboratory in packaging and shipping
    - Must complete a training program in packaging and shipping of biological substances
    - Documentation of completion of a certified training program
    - Check your state for training programs

The following chart illustrates some key shipping and packaging requirements. However, each institution must have a detailed protocol, and individuals who have completed certified training in packaging and shipping

Key Points For Packaging and Shipping Infectious and Diagnostic Specimens

- Must be triple packed
- Containers must be burst-proof, leak-proof, and absorbent
  - Primary receptacle
    - water tight and/or sift proof
    - must withstand pressure and temperature changes without leakage during normal transport (ground or air)
  - Secondary receptacle
    - water tight, with absorbent material between primary and secondary containers
    - must withstand pressure and temperature changes without leakage during normal transport (ground or air)
  - Outer package
    - durable material
    - labeled with the UN Specification marking noting year and location made, and manufacturer number
    - infectious and diagnostic substances must be identified with an IS or DS label with the UN2814 code
- Completion of Shippers Declaration for Dangerous Goods
  - IATA Shippers Declaration of Dangerous Goods

NOTE:
UN Packaging Codes

- Established by the United Nations Committee of Experts on the Transportation of Dangerous Goods
- A uniform international system for identifying and packaging Class 3, 4, 5, 6.1, 8 and 9 dangerous goods for transport
- UN Committee has assigned all dangerous goods to one of three Packing Groups:
  - Packing Group I (high danger)
  - Packing Group II (medium danger)
  - Packing Group III (low danger)

Additional readings and references on packaging and shipping requirements:

CDC: Packaging and Shipping of Biomedical Material

International Air Transport Association (IATA)

Transportation and Transfer of Biological Agents

Specimen Collection and Transportation Guidelines

In dealing with bioterrorism agents, packaging and shipping are critical safety issues.

Specimen Collection & Transport Guidelines

Specimen Collection for Suspected Bioterrorism Agents

Bioterrorism Agents / Diseases

The CDC has classified potential bioterrorism agents into three categories:

Category A Agents Category B Agents Category C Agents

Definition of Category A Agents

Category A Agents are high-priority agents that pose a risk to national security. Agents are classified as Category A if they meet the following criteria:

- **Can be easily disseminated or transmitted from person to person**
- **Result in high mortality rates and have the potential for major public health impact**
- **Might cause public panic and social disruption**
- **Require special action for public health preparedness**

Category A Pathogens:

Below is the CDC's list of Category A Agents. These organisms will be discussed in more detail later.

Bacillus anthracis
Clostridium botulinum toxin
Yersinia pestis
Smallpox
Francisella tularensis
Viral hemorrhagic fever agents

Definition of Category B Agents

Category B Agents are the second highest priority agents and include those that:

- **Are moderately easy to disseminate**
- **Result in moderate morbidity rates and low mortality rates**
- **Require specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance**

Category B Pathogens:

The list of Category B Agents is quite extensive and includes the following organisms:

Brucellosis - Brucella spp.
Epsilon toxin of *Clostridium perfringens*
Food safety threats (e.g., *Salmonella species, Escherichia coli O157:H7, Shigella*)
Glanders (*Burkholderia mallei*)
Melioidosis (*Burkholderia pseudomallei*)
Psittacosis (*Chlamydia psittaci*)
Q fever (*Coxiella burnetii*)
Ricin toxin from *Ricinus communis* (castor beans)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])
Water safety threats (e.g., *Vibrio cholerae, Cryptosporidium parvum*)

Definition of Category C Agents

Category C Agents are the third highest priority agents and include emerging pathogens that could be engineered for mass dissemination in the future due to:

- **Availability**
- **Ease of production and dissemination**
- **Potential for high morbidity and mortality rates and major health impact**

Category C Pathogens:

Category C Agents include emerging infectious diseases such as:

Nipah Virus
Hantavirus

CDC's TOP LIST OF SELECTED AGENTS:
Includes both Category A and Category B Agents

- Anthrax - *Bacillus anthracis*
- Botulism - *Clostridium botulinum* toxin
- Plague - *Yersinia pestis*
- Viruses - Smallpox (Variola virus), *Ebola*
- Tularemia - *Francisella tularensis*
- *Brucellosis - Brucella spp.*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*

Category A Pathogens:

Bacillus anthracis

Causative agent of anthrax

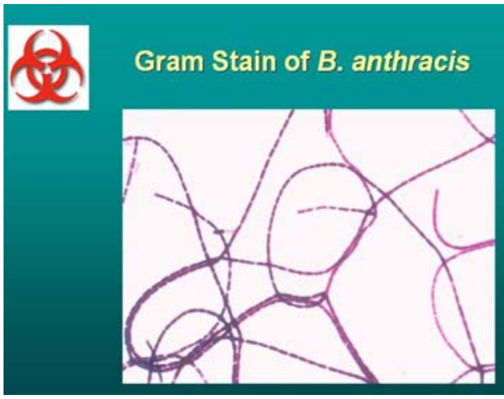
MICROBIOLOGY:

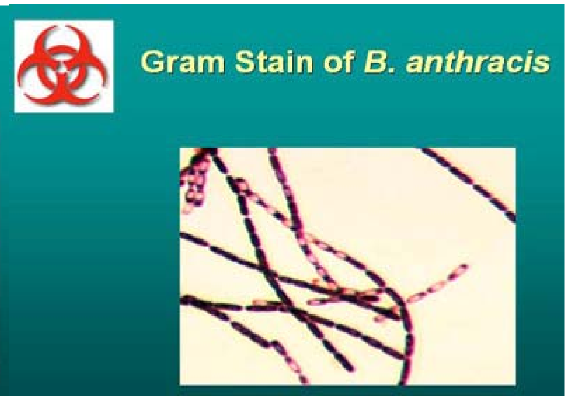
Morphology:

- Large gram positive bacillus (1-1.5 x 3-5 microns)
- Appears in single, short or long chains
- May become gram variable after 72 hours
- Non-hemolytic
- Flat-slightly convex
- "Medusa head" or "Comet tail" colonies on Sheep Blood Agar (SBA)
- Grey, "ground glass" appearance
- Irregular edges with comma shaped projections
- Tenacious consistency; stands up when teased with a loop

- Use routine plating media: blood agar, CHOC, broth (not THIO)
- Incubation in ambient air (better than CO_2 which might inhibit spore production)

- Rapid growth may occur - within 8 hours





Bacillus anthracis Technical Clues:

- **Large gram-positive, aerobic rods**
- **Rapidly growing, flat, "ground-glass" colonies on SBA**
- **Non-hemolytic**
- **Non-motile**
- **Catalase positive (CAUTION: test produces aerosol)**

PATHOGENESIS:

- Spore-forming gram positive bacilli
- Infectious form is the spore
- Spores are resistant to drying, heat, ultraviolet light, gamma radiation and to many disinfectants
- Can therefore remain viable for years in soil, water, and under direct sunlight
- Animals eat grass from infected soil
- Humans acquire infection from contact with anthrax infected animals or animal products
- Once the spores enter the body through abrasions, inhalation or ingestion, they are phagocytized by macrophages and carried to the regional lymph nodes
- At this point can gain access to the blood stream and disseminate rapidly throughout the body
- Person to person transmission in unlikely

A zoonotic disease in three forms:

- Cutaneous
- Inhalation
- Gastrointestinal

CLINICAL FEATURES:

Cutaneous anthrax

- Most common form - more benign, usually non-fatal
- Severe cases up to 25% mortality
- Presents as a nondescript, painless papule three to five days following exposure
- Lesions most commonly appear on the head, neck and extremities
- The papule evolves from a vesicular to a pustular stage with central necrosis and formation of a black eschar within one to two days

http://www.bt.cdc.gov/agent/anthrax/anthrax-images/cutaneous.asp

Inhalation anthrax

- Most severe form - high mortality
- Incubation period of up to six weeks
- Incubation period is related to the quantity of spores inhaled
- "Woolsorter's Disease" (sheep, goats, cattle)
- Begins as a non-specific "flu-like" illness
- Symptoms include fever, nonproductive cough, myalgia, malaise, chest discomfort
- Within a few days, may progress to shortness of breath, wheezing, cyanosis - abrupt onset of respiratory failure (2-4 days)
- Patients experience shock, chest wall edema, meningitis
- Once pneumonia develops, mortality is 100%
- Death may occur rapidly

This form associated with Bioterrorism threat

- Well suited as a bioweapon because it is capable of forming hardy spores that can remain stable for decades
- Spores are invisible, odorless and tasteless
- Amount smaller than a speck of dust can make a person ill
- Deadliest form of this disease occurs when the spores are inhaled and germinate in the lungs
- Death can be rapid

http://www.bt.cdc.gov/agent/anthrax/anthrax-images/inhalational.asp

Gastrointestinal anthrax

- Least common form - may be fatal, toxemia and sepsis
- Presents in two forms: Oropharyngeal and Classic gastrointestinal disease

Oropharyngeal:

- Cervical edema and regional lymphadenopathy with concomitant dysphagia and respiratory difficulties

Classic gastrointestinal disease:

- Nausea, vomiting, malaise progressing to bloody diarrhea and abdominal distention due to massive ascites and sepsis
- Occurs through ingestion of spore infected meat

GI anthrax usually occurs in countries where vaccination of animals does not occur

***Bacillus anthracis* can be found in:**

- **Direct specimen smear**
- **Blood culture or CSF (inhalation)**
- **Vesicle fluid, swab or biopsy or eschar (cutaneous)**
- **Stool (gastrointestinal)**

SPECIMEN COLLECTION:

Cutaneous anthrax:

- Vesicular stage: collection by sterile swab using aseptic technique
- Eschar stage: gently lift outer edge of scab, slowly rotate swab beneath edge

- Swabs should be transported directly to the lab at room temperature
- If transport exceeds one hour, swabs should be transported at 2-8^{0}C

Inhalation anthrax:

- Blood culture
- Sputum specimen

Gastrointestinal anthrax:

- Blood culture
- Stool specimen

Organisms will grow in Bactec and BacT alert systems

SAFETY:

- Considered a BSL-2 organism for processing clinical specimens
- Requires BSL-3 practices for culture manipulation that produce aerosols
- Environmental samples suspected of containing Bacillus anthracis should not be processed in the clinical lab due to the potential aerosol release of spores
- Environmental samples are high risk and should be sent to the appropriate authorities

REPORTING:

- Send isolates to the appropriate authorities

Send isolates to the Public Health Lab for confirmation if organism is:

- A large gram positive bacillus
- Non-hemolytic
- Non-motile
- Catalase positive
- Spores produced when cultured aerobically without CO_2

TREATMENT:

- Preventable by vaccine, antibiotic prophylaxis, or early treatment before pneumonia develops
- Penicillin, Ciprofloxacin or Doxycycline

Please visit the CDCs website for additional information on Anthrax:

http://www.bt.cdc.gov/agent/anthrax/index.asp

Clostridium botulinum toxin

Causative agent of botulism
Spores of *Clostridium botulinum* are ubiquitous in soil and water

MICROBIOLOGY:

Morphology:

- Gram positive, straight rods occurring singly or in pairs
- Anaerobic spore forming bacteria
- Spores usually subterminal
- Grows on anaerobic blood media producing gray-white colonies
- Colonies appear circular to irregular in shape
- Usually beta-hemolytic

PATHOGENESIS:

- Absorbed through the mucous membranes of the respiratory tract or through the wall of the gastrointestinal tract
- Enters the blood stream and binds irreversibly to the peripheral nervous system
- Signs and symptoms begin after 12-36 hours
- Mortality rates range from 5-10%
- No person to person transmission

Three main types of botulism:

- Food-borne botulism
- Infant botulism
- Wound botulism

CLINICAL FEATURES:

Food-borne botulism

- Food-borne disease most common
- Occurs when a person ingests preformed toxin in contaminated food
- Leads to illness in a few hours to days
- Symptoms begin within 6 hours to 2 weeks after exposure (usually within 12-36 hours)
- Toxin-mediated disease
- Potent neurotoxin, contaminates food
- Home-processed foods, rather than commercially processed foods, have been involved in most of the outbreaks

Infant botulism

- Infants ingest spores, but not preformed toxin, from soil, household dust, honey, or another source
- Results from *in vivo* multiplication of *C. botulinum* with production of the neurotoxin within the infant gut
- Clinical features include constipation, listlessness, difficulty in sucking and swallowing, an altered cry, hypotonia, and muscle weakness
- Eventually baby appears "floppy", loses head control, and may develop ptosis, ophthalmoplegia, flaccid facial expression, dysphagia, and other neurologic signs
- Respiratory arrest or respiratory insufficiency necessitating respiratory therapy occur
- Small number of infants with laboratory-confirmed infant botulism have died

Wound botulism

- Occurs when wounds are infected with *C. botulinum* that secretes the toxin
- Results from production of botulinal toxin *in vivo* after *C. botulinum* has multiplied in an infected wound

CLINICAL FEATURES:

Initial symptoms:

- Cranial neuropathies
- Dry mouth, blurred vision, double vision, drooping eyelids, photophobia

More serious cases:

- Classic descending flaccid paralysis and muscle weakness
- Death by respiratory failure
- Paralysis of muscles of tongue, pharynx, intercostal muscles or diaphragm
- About 5% die
- Recovery takes months
- Those who survive have fatigue and shortness of breath for years

SPECIMEN COLLECTION

CDC Guidelines:

Enema fluid:

- Collect 20 cc of fluid
- Purge with a minimum of sterile, non-bacteriostatic water
- Transport at 4^0C

Food sample:

- Collect 10-50 grams
- Submit food in original container
- Place each container into leak-proof, sealed transport device
- Transport at 4^0C

Nasal swab:

- For aerosolized release, collect nasal swabs for *C. botulinum*, serum for toxin
- Transport room temperature

Serum:

- Collect 10cc of serum
- Transport at 4^0C

Stool:

- Collect 10-50 grams of stool into a sterile, leak-proof container
- Transport at 4^0C

Other:

- In an intentional release, collecting environmental surface samples on swabs may be useful
- Transport at 4^0C

SAFETY:

- Very dangerous toxin to handle
- Exposure is primary laboratory safety concern
- BSL-2 practices, equipment, clothing, and facilities are recommended for the handling of all suspected materials
- BSL-3 practices for manipulation of specimens

REPORTING:

Role of laboratory:

- Do not culture
- Notify State and Public Health labs immediately for advisement

BIOTERRORISM THREAT

- Inhalation form may occur
- Incubation period is 24-72 hours if inhaled
- Powerful neurotoxin
- Exerts its effect by interrupting nerve cell function
- Toxin is released when the bacterial cell lyses

Clostridium botulinum is the most toxic substance in the world

- 100,000 times more toxic than sarin nerve gas
- Toxins can be ingested, inhaled, or injected to exert their deadly effect
- Toxins can be delivered as an aerosol
- People who inhale toxins can go into respiratory paralysis

If aerosolized: 1 gram can kill 1.5 billion people

TREATMENT:

- Antitoxin available
- Respiratory failure and paralysis that occur with severe botulism may require a patient to be on a breathing machine (ventilator) for weeks, plus intensive medical and nursing care
- After several weeks, the paralysis slowly improves
- If diagnosed early, foodborne and wound botulism can be treated with an antitoxin which blocks the action of toxin circulating in the blood
- This can prevent patients from worsening, but recovery still takes many weeks
- Physicians may try to remove contaminated food still in the gut by inducing vomiting or by using enemas
- Wounds should be treated, usually surgically, to remove the source of the toxin-producing bacteria
- Good supportive care in a hospital is the mainstay of therapy for all forms of botulism
- Currently, antitoxin is not routinely given for treatment of infant botulism

Please visit the CDCs website for additional information on Botulism:

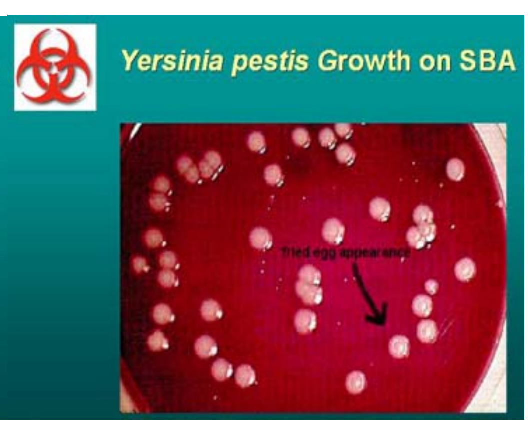
http://www.bt.cdc.gov/agent/botulism/index.asp

Yersinia pestis

Causative agent for the Plague

MICROBIOLOGY:

- Plump gram negative bacilli - family Enterobacteriacea (1.0-2 x 0.5 microns)
- Bipolar staining with Wayson or Wright-Giemsa stain, occasionally with Gram Stain
- May be seen on direct exam of specimen
- Gray-white pinpoint colonies at 24 h on SBA; slightly yellow and opaque after 48 h
- Raised, irregular "fried egg and/or "hammered copper" shiny surface appearance after 48-72 h
- Non-hemolytic on BAP
- Non-lactose fermenting
- Non-motile
- Indole negative
- Oxidase negative
- Urease negative
- Catalase positive (CAUTION: test produces aerosol)
- Most carbohydrates negative
- Flocculent growth in BHI
- K/A reaction in TSI
- Routine plating media and BHI broth (two sets for incubation)
- Lower respiratory specimens (CIN media - selective for contaminated specimens; pink colonies)
- Optimal growth at 28^{0}C or room temperature
- Slow growing at 35-37^{0}C



Yersinia pestis Technical Clues:

- **Gram-negative rods from blood, lymph node aspirate, or respiratory specimens**
- **Colonies resemble enterics, but grow better at 28^0C than at 35^0C**
- **Non-lactose fermenter on MAC**
- **Catalase positive**
- **Oxidase negative**
- **Urease negative**
- **Indole negative**

PATHOGENESIS:

Virulence factors:

- Extremely pathogenic
- The ability to absorb organic iron
- The presence of coagulase and fibrinolysin
- Endotoxin
- Antiphagocytic protein F1
- A 45-mD plasmid that encodes for virulence factors V and W

- Occurs naturally in rodents - rats, squirrels, prairie dogs
- Endemic in western part of the U.S., west of the Rockies
- If found in the east - notify Infectious Disease doctors
- Humans acquire infection through bites of infected fleas
- Human to human transmission is uncommon
- Occurs only in pneumonic form through respiratory droplets

- In the event of bioterrorism, *Yersinia pestis* would likely be spread by aerosol release causing outbreaks of respiratory illness 1 to 6 days following

Three forms of the plague:

- Pneumonic
- Bubonic

- Septicemic

CLINICAL FEATURES:

Pneumonic Plague:

- Occurs when *Y. pestis* infects the lungs
- Can be spread person to person through air
- Transmission can take place if someone breathes in aerosolized bacteria, which could happen in a bioterrorist attack
- Pneumonic plague also spread by breathing in *Y. pestis* suspended in respiratory droplets from a person (or animal) with pneumonic plague
- Usually requires direct and close contact with the ill person or animal
- Pneumonic plague may also occur if a person with bubonic or septicemic plague is untreated and the bacteria spreads to the lungs

- Incubation 2-3 days
- Fever, headache, hemoptysis, respiratory failure, chest pain, cough, dyspnea
- Diagnosis made by biopsy or routine culture
- Patients die due to respiratory failure and circulatory collapse
- Patients must be isolated for 72 hours after starting antibiotics

Bubonic Plague:

- Most common form of plague
- Occurs when an infected flea bites a person or when materials contaminated with *Y. pestis* enter through a break in a person's skin
- Patients develop swollen, tender lymph glands (called buboes) and fever, headache, chills, malaise and weakness 2-8 days following flea bite
- Septicemia and shock

- Bubonic plague does not spread from person to person
- Incubation 2-10 days

Septicemic Plague:

- Occurs when plague bacteria multiply in the blood
- Can be a complication of pneumonic or bubonic plague or can occur by itself
- When it occurs alone, it is caused in the same ways as bubonic plague; however, buboes do not develop
- Fever, chills, prostration, abdominal pain, shock, and bleeding into skin and other organs
- Disseminated intravascular coagulation, tissue necrosis, peripheral gangrene (black death)
- Black fingers, toes, and nose

- Septicemic plague does not spread from person to person

SPECIMEN COLLECTION:

Samples for analysis:

- Blood cultures
- Bubo aspirates
- Sputum
- CSF
- Scrapings from skin lesion

Blood cultures:

- Room temperature transport

Sputum:

- Transport at refrigerated temperatures if time exceeds one hour

Bubo aspirate:

- Inject 1 ml sterile saline into bubo
- Aspirate several times until blood tinged
- Transport capped syringe immediately to lab

- Inoculate samples on routine lab media immediately
- Direct Fluorescent Antibody test for further confirmation

SAFETY:

- Considered a BSL-2 organism for processing clinical specimens
- Requires BSL-3 practices for culture manipulations that might produce aerosols

REPORTING:

Send preliminary isolates to state

Isolates should be sent to a public health lab for confirmation if:

- Gram negative bacillus
- Grows at 35-37^{0}C; faster at room temperature
- Catalase positive
- Non-motile (37^{0}C and room temperature)
- Oxidase negative

TREATMENT:

- To reduce the chance of death, antibiotics must be given within 24 hours of first symptoms
- Streptomycin, gentamicin, the tetracyclines, and chloramphenicol are all effective against pneumonic plague
- Antibiotic treatment for 7 days will protect people who have had direct, close contact with infected patients
- Post-exposure prophylasis with doxycycline or ciprofloxacin
- Wearing a close-fitting surgical mask also protects against infection
- A plague vaccine is not currently available for use in the United States

Please visit the CDCs website for additional information on the Plague:

http://www.bt.cdc.gov/agent/plague/index.asp

VARIOLA
SMALL POX VIRUS

MICROBIOLOGY:

- Poxviridae family
- Large DNA virus
- Largest and most complex of all viruses
- Dumb-bell shaped core
- Complex membranes

PATHOGENESIS:

Serious, contagious and sometimes fatal disease

- Initial smallpox infection site is the respiratory tract
- Virus multiplies in the lymph nodes, enters the blood stream
- First causes lesions in mouth, upper and lower airways
- Skin lesions occur first on face and head, followed by the trunk, then the extremities
- Smallpox derived from Latin word for "spotted" and refers to the raised bumps that appear on the face and body of an infected person

Transmitted by:

- Person to person contact

- Droplet nuclei
- Dust
- Fomites (contaminated clothing)
- Can be spread through ventilation systems within buildings

Two clinical forms of Smallpox:

- Variola Major
- Variola Minor

CLINICAL FEATURES:

Variola Major:

- Acute viral illness
- Severe and most common form of smallpox
- More extensive rash and higher fever
- Severe morbidity in non-immune population

Four types of variola major smallpox:

- Ordinary (the most frequent type, accounting for 90% or more of cases)
- Modified (mild and occurring in previously vaccinated persons)
- Flat (rare and very severe)
- Hemorrhagic (rare and very severe)

- Historically, variola major has an overall fatality rate of about 30%; however, flat and hemorrhagic smallpox usually are fatal

Symptoms:

- Sudden onset of fever, severe headache, vomiting, chills, backaches, flu-like illness
- After 2-4 days rash develops which quickly progresses to pustules by day 5 or 6 (rapidly progressive skin lesions)
- Most prominent on face and extremities
- Day 9, pustules reach largest size, then start to scab over and slough off after day 20
- Permanent scars are common
- If patient survives, alot of disfigurement

- Early stages resemble chicken pox or allergic-contact dematitis

- Person to person transmission likely - contact with lesions
- Incubation 7-17 days (12 days)
- Patient infectious once rash appears and through separation of scabs
- Patient and contacts are quarantined for about 16 days after exposure

SPECIMEN COLLECTION:

CDC GUIDELINES:

Biopsy:
Aseptically place 2-4 portions of tissue into a sterile, leak-prook, freezable container
Transport in less than 6 hours at 4^0C
Store at -20 to -70^0C

Scabs:
Aseptically place scrapings/material into a sterile, leak-proof, freezable container
Transport in less than 6 hours at 4^0C
Store at -20 to -70^0C

Vesicular fluid:
Collect fluid from separate lesions onto separate sterile swabs
Be sure to include cellular materials from the base of each respective vesicle
Transport in less than 6 hours at 4^0C
Store at -20 to -70^0C

SAFETY:

- Highly infectious
- Must contact State Lab for appropriate specimen and handling
- Requires BSL-4
- Maximum Containment Lab (CDC)

REPORTING:

- Diagnosis confirmed by cell culture and electron microscopy for demonstrating viral particles in fluids, scabs and tissues
- DO NOT process any specimen from a patient suspected to have Smallpox
- Notify state public health lab immediately
- Clinical labs are responsible for the notification, advising medical staff on specimen selection, packing and shipping

BIOTERRORIST THREAT:

- 1980 World Health Organization declared that smallpox was globally eradicated
- Children stopped receiving vaccinations
- Military stopped vaccination programs in 1989
- Good portion of population and military are now susceptible
- If a case is reported must suspect biological attack
- Confirmed case of smallpox is an international emergency and needs to be reported

to public health officials immediately

- Only persons vaccinated within the past 3 years are considered immune
- If vaccination is given within 7 days of exposure, the disease can be prevented
- No effective anti-viral drugs at this time

TREATMENT:

Vaccine available: Refer to the CDC's website for additional information

http://www.bt.cdc.gov/agent/smallpox/vaccination/facts.asp

Please visit the CDCs website for additional information on Smallpox:

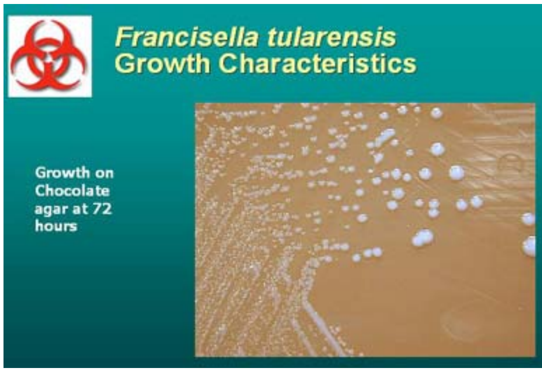
http://www.bt.cdc.gov/agent/smallpox/index.asp

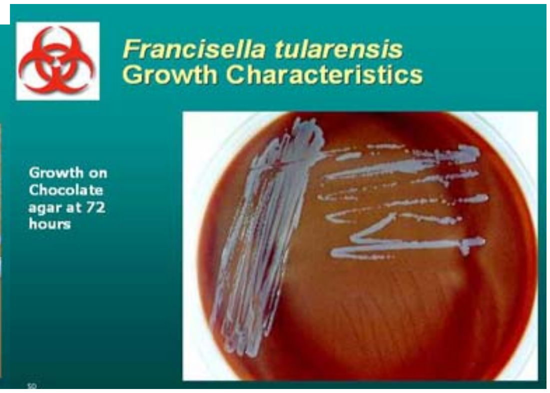
Fransicella tularensis

Causative agent of tularemia

MICROBIOLOGY:

- Facultative intracellular coccobacilli
- Faint staining, very thin, gram negative coccobacilli
- Appear as single cells (0.2-0.5 x 0.7-1.0 microns)
- Grows poorly on SBA; cysteine required for growth
- Grows slowly at 35-37^{0}C on SBA
- Gray-white colonies (48-72 hours) - usually too small to be seen at 24 h
- Smooth, shiny, and butyrous colonies
- Does not require CO_2
- Does not grow on MacConkey or EMB agar
- Slow growth in broth
- Catalase negative, or weakly positive
- Non-hemolytic
- Non-motile
- Oxidase negative
- Urease negative
- X and V factors are not required (negative)





Francisella tularensis Technical Clues:

- **Tiny, gram-negative coccobacilli from blood, lymph node aspirate, or respiratory specimens (or skin ulcer)**
- **Isolates growing slowly on chocolate agar, poorly or not at all on blood agar at 72 h**
- **Oxidase negative**
- **Catalase (weak positive) (CAUTION: test produces aerosols)**
- **XV or satellite negative**
- **Beta lactamase positive**

Other Similar Gram-Negative Coccobacilli:

Most likely:

- ***Acinetobacter* (oxidase negative)**
- ***Actinobacillus***
- ***Haemophilus aphrophilus***
- ***Bordetella*, Group IV (inert, urea positive)**
- ***Pasturella* (nonsticky, MAC positive)**

Least likely:

- **DF-3**
- ***Brucella* (urea positive in seconds to minutes)**

PATHOGENESIS:

- Zoonotic disease
- One of the most infectious pathogens
- Can survive in water, carcasses, hides and for years in frozen rabbit meat

- Organism infects a variety of animal hosts
- Small mammals (rabbits, squirrels, mice, voles) by insect vectors
- Humans acquire infection from environmental exposures
- Occurs after intradermal, respiratory or gastric inoculations

- Facultative intracellular bacterium capable of multiplying within macrophages
- Once inoculated, will multiply quickly and move to regional lymph nodes

- Initial host response - intense polymorphonuclear suppuration
- Later - granulomatous inflammation, characterized by multinucleated giant cells

- Particularly attractive biothreat agent
- As few as 10 organisms are capable of causing disease
- Septicemic tularemia form most probable form for terrorist attacks

Clinical Syndromes:

- Ulceroglandular tularemia
- Glandular tularemia
- Oculoglandular tularemia
- Typhoidal tularemia
- Pneumonic tularemia

CLINICAL FEATURES:

People can acquire tularemia many different ways:

- Through bite of an infected insect or other arthropod (usually a tick or deerfly)
- Handling infected animal carcasses
- Eating or drinking contaminated food or water
- Breathing in *F. tularensis*
- Incubation 2-10 days

Ulceroglandular tularemia:

- Red, painful papule progresses to ulcer with red raised border
- Enlarged, tender localized lymphadenopathy

Glandular tularemia:

- Tender adenopathy in absence of cutaneous lesion

Oculoglandular tularemia:

- Organisms gain entry via conjunctivae
- Lid edema and painful conjunctivitis with yellow conjunctival ulcers or papules
- Associated painful regional lymphadenopathy

Typhoidal tularemia:

- Fever, chills, headache, myalgias, abdominal pain, nausea, vomiting, diarrhea and cough

Pneumonic tularemia:

- Fever, cough with scant sputum production
- Substernal tightness

- Pleuritic chest pain
- Biothreat form - high mortality

- Tularemia is not known to be spread person to person so infected individuals do not need to be isolated

SPECIMEN COLLECTION:

Blood cultures

Sputum

- Transport at refrigerated temperatures if time exceeds one hour

Lymph node aspirates

- Insert 20 gauge needle into node and collect any purulent material
- Transport capped syringe immediately to lab

SAFETY:

- Dangerous, highly virulent organism
- Should not be manipulated at the bench
- Laboratory acquired infections can occur easily

- Considered BSL-2 for processing clinical specimens
- Requires BSL-3 practices for culture manipulations that might produce aerosols

BIOTERRORISM AGENT:

- *Francisella tularensis* is highly infectious
- Small number of bacteria (10-50 organisms) can cause disease
- If *F. tularensis* were used as a bioweapon, the bacteria would likely be made airborne for exposure by inhalation
- Persons who inhale an infectious aerosol would generally experience severe respiratory illness, including life-threatening pneumonia and systemic infection, if they were not treated
- The bacteria that cause tularemia occur widely in nature and could be isolated and grown in quantity in a laboratory, although manufacturing an effective aerosol weapon would require considerable sophistication

TREATMENT:

- Acute onset of disease occurs from 2-10 days after exposure
- Treated with intramuscular streptomycin for 10-14 days or gentamicin
- Tetracycline and chlorampheicol are also used

- Vaccine for tularemia is under review by the Food and Drug Administration and is not currently available in the United States

Please visit the CDCs website for additional information on Tularemia:

http://www.bt.cdc.gov/agent/tularemia/index.asp

Viral Hemorrhagic Fever Agents

Members of:

Arenavirus family

- Lassa
- Junin

Filovirus family

- Ebola
- Marburg

MICROBIOLOGY:

Lassa Virus:

- Enveloped, irregular-shaped capsid containing a two-segmented, single-stranded RNA genome
- Diagnosis: Serology, PCR

Ebola and Marburg Virus:

- Enveloped, long, filamentous and irregular capsid forms with single-stranded RNA
- Diagnosis: Electron microscopy, cell culture in monkey kidney cells

PATHOGENESIS:

- Agents of Viral Hemorrhagic Fever (VHF) infect human cells such as macrophages causing cellular and vascular damage and death
- Severe multi-system syndrome
- Target vascular system and cause changes in vascular permeability which leads to microvascular damage
- Overall vascular system is damaged
- Body's ability to regulate itself is impaired
- Symptoms quickly evolve to shock with mucous membrane hemorrhage
- Renal failure accompanies cardiovascular decline
- Causes serious human disease

- Frequent fatal cases
- Up to 88% mortality
- Airborne contact
- Person to person contact occurs
- Unstable virus

- Found in Africa and South America
- May be transported worldwide

CLINICAL FEATURES:

Lassa Fever:

- Incubation period 5-7 days
- Sore throat, lower back pain, eye symptoms
- Progress to nausea, vomiting, diarrhea, cough, respiratory tract complication, occasionally hemorrhage

Ebola and Marburg Virus:

- Incubation period of 4-16 days
- Very ill with flu-like symptoms
- Progress to nausea, vomiting, diarrhea, rash
- Gastrointestinal bleeding, other hemorrhages, with shock occurring shortly before death

SPECIMEN COLLECTION:

Diagnosis of VHF is made by clinical findings and confirmed by serologic testing

Serum:

- Collect 10-12 cc of serum (additional specimen handling protocols are still under development)
- Transport in less than 2 hours at room temperature
- Store at less than 4^0C

SAFETY:

- VHF viruses are highly infectious
- Avoid manipulation of specimen material
- Use BSL-4 practices
- Clinical labs should not attempt to culture or perform any diagnostic assays on specimens suspected of containing VHF agents

TREATMENT:

- Patients receive supportive therapy, but generally speaking, there is no other

treatment or established cure for VHFs
- Ribavirin, an anti-viral drug, has been effective in treating some individuals with Lassa fever
- No vaccines exist that can protect against these diseases

Please visit the CDCs website for additional information on VHFs:

http://www.bt.cdc.gov/agent/vhf/index.asp

CATEGORY B AGENTS

Brucellosis - Brucella spp.
Epsilon toxin of *Clostridium perfringens*
Food safety threats (e.g., *Salmonella species, Escherichia coli O157:H7, Shigella*)
Glanders (*Burkholderia mallei*)
Melioidosis (*Burkholderia pseudomallei*)
Psittacosis (*Chlamydia psittaci*)
Q fever (*Coxiella burnetii*)
Ricin toxin from *Ricinus communis* (castor beans)
Staphylococcal enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis])
Water safety threats (e.g., *Vibrio cholerae*, *Cryptosporidium parvum*)

The above Category B agents are all potential biological weapons which can be used in a biological attack. Brucellosis and Burkholderia will be the only agents covered in this section. These two organisms are considered select agents and their details will be discussed below. The CDC has additional information on some of the above organisms.

http://www.bt.cdc.gov/Agent/agentlist.asp#categorycdiseases

BRUCELLA spp.

Causative agent of Brucellosis

Brucella species, usually *B. abortus* (cattle), *B. melitensis*, *B.ovis* (sheep, and goats), *B. suis* (pigs), and rarely *B. canis* (dogs)

MICROBIOLOGY:

- Tiny, faintly staining, gram negative coccobacilli from blood or bone marrow **(0.4 x 0.8 microns)**
- **May stain gram-positive**
- **Organisms will grow in routine blood culture systems, but may require extended incubation**
- Will grow on SBA and CHOC
- **Light "dust" of growth after 24 h**
- 2-3 days for colony appearance
- **Smooth, convex, and raised with an entire edge on CHOC and SBA after 48 h**
- Non-motile
- **Non-hemolytic**
- Oxidase positive
- Urease positive
- **Catalase positive**
- Many isolates require supplementary CO_2 for growth, especially on primary isolation

Brucella spp. Technical Clues:

- **Tiny, faintly staining, gram-negative coccobacilli from blood, bone marrow, or lymphoid tissue**
- **Slow growth on SBA, CHOC (2-3 days for colonies to appear)**
- **Oxidase positive**
- **Urease positive**
- **Catalase positive**
- **No satellite phenomenon (XV factors)**

Other Similar Gram-Negative Bacilli:

- ***Achromobacter* Group B**
- ***Acidovorax* spp.**
- ***Agrobacterium* spp.**
- ***Chryseobacterium* spp.**
- ***Methylobacterium* spp.**
- ***Ochrobactrum* spp.**
- ***Pseudomonas* spp.**
- ***Riemerella***
- ***Roseomonas* spp.**

CLINICAL FEATURES:

- Extremely variable
- In the acute form (<8 weeks from illness onset), symptomatic, nonspecific and "flu-like," including fever, sweats, malaise, anorexia, headache, myalgia, and back pain
- In the undulant form (<1 year from illness onset), symptoms include undulant fevers, arthritis, and orchiepididymitis in males
- Neurologic symptoms may occur acutely in up to 5% of cases
- In the chronic form (>1 year from onset), symptoms may include chronic fatigue syndrome-like, depressive episodes, and arthritis

INCIDENCE:

- In the United States: < 0.5 cases per 100,000 population
- Most cases are reported from California, Florida, Texas, and Virginia

SEQUELAE:

Extremely variable including:

- Granulomatous hepatitis
- Peripheral arthritis
- Spondylitis
- Anemia
- Leukopenia
- Thrombocytopenia
- Meningitis
- Uveitis
- Optic neuritis
- Papilledema
- Endocarditis

TRANSMISSION:

- Zoonotic
- Commonly transmitted through abrasions of the skin from handling infected mammals
- In the United States, occurs more frequently by ingesting contaminated milk and dairy products
- Highly infectious in the laboratory via aerosolization; cultures are considered to warrant biosafety level-3 precautions
- Direct person-to-person spread of brucellosis is extremely rare

REPORTING:

Brucellosis is a nationally notifiable disease and reportable to the local health authority

TREATMENT:

- Treatment can be difficult
- Doctors can prescribe effective antibiotics
- Usually, doxycycline and rifampin are used in combination for 6 weeks to prevent reoccurring infection
- Depending on the timing of treatment and severity of illness, recovery may take a few weeks to several months
- Mortality is low (<2%), and is usually associated with endocarditis

Please visit the CDCs website for additional information on Brucella:

http://www.bt.cdc.gov/agent/brucellosis/index.asp

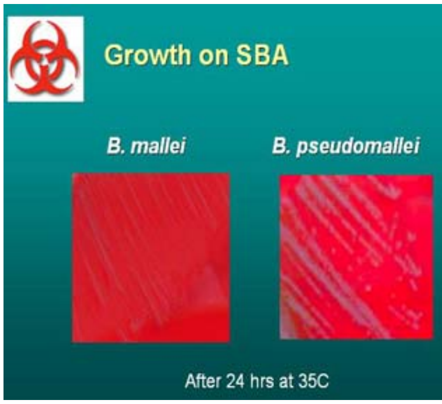
Burkholderia spp.

Select organisms include *Burkholderia mallei* causative agent of **Glanders** and *Burkholderia pseudomallei* causative agent of **Meliodosis**.

MICROBIOLOGY

	Burkholderia mallei	Burkholderia pseudomallei
Gram Stain Reaction	**Gram negative coccobacillus**	**Gram negative bacillus (suggestion of bipolar staining)**
Media Requirements	**Will grow on SBA and CHOC, possibly on MAC producing mature (small) colonies at 72 hours**	**Will grow on SBA, CHOC, MAC producing mature colonies at 48 to 72 hours**
Oxidase	**Variable**	**Positive**
Indole	**Negative**	**Negative**
Colistin	**Resistant**	**Resistant**
Catalase	**Positive (CAUTION test produces aerosol)**	**Positive (CAUTION test produces aerosol)**
Motility	**Non-motile**	**Motile**
TSI	**No change, slant or butt**	**No change, slant or butt, or acid over no change**
Arginine Dihydrolase	**Positive**	**Positive**

Nitrate Reduction	Reduces Nitrate without gas	Reduces Nitrates to gas



Burkholderia pseuodmallei causes Meliodosis, an aggressive granulomatous pulmonary disease caused by ingestion, inhalation, or inoculation of the organism with further metastatic abscess formation in the lungs and other viscera.

Burkholderia mallei causes Glanders, a destructive and contagious bacterial disease of animals which can be transmitted to humans. It is characterized by nodular lesions of the lungs and other organs as well as ulcerative lesions of the skin and mucous membranes of the nasal cavity and respiratory passages. The disease typically has a progressive course and poses a significant human health risk.

For an extensive overview of Burkholderia please read the following article:

http://www.emedicine.com/emerg/topic884.htm

***Burkholderia pseudomallei* Technical Clues:**

- **Gram negative aerobic rods**
- **Slow growth on SBA and MAC**
- **Oxidase positive**

- **Catalase positive**
- **Motile**

***Burkholderia mallei* Technical Clues:**

- **Gram-negative coccobacilli**
- **Very slow growth on SBA and little if any growth on MAC**
- **Oxidase variable**
- **Catalase positive**
- **Non-motile**

Other Similar Gram-Negative Bacteria:

- ***Burkholderia cepacia***
- ***Burkholderia gladiolii***
- ***Pseudomonas mendocina***
- ***Pseudomonas stutzeri***
- ***Ralstonia picketii***
- ***Stenotrophomonas maltophilia***

"All images in this section were provided by CDC"

SUMMARY:

Clinical laboratory scientists and microbiologists have been educated about the agents mentioned above. Most microbiologists in their professional career have never isolated some of the above agents such as *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, VHF, etc., and at one time may have thought they never would. Unfortunately the time is upon us NOW with the U.S. at war with the middle east. Biological and Chemical agents are a sure thing. The correct statement is not "if this will happen" but "when it will happen." Do you feel the U.S. is ready to fight biological and chemical warfare? Do you as a laboratory professional feel prepared at your institution to handle a terroristic attack? Do you feel your immediate laboratory supervisor has an appropriate plan in place that will enable you to respond efficiently and appropriately should a biological agent find its way into your laboratory? These are questions you need to address with your colleagues and administrators at your institution.

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Interent Sites

Biological Agents
http://www.bt.cdc.gov/Agent/agentlist.asp#categorycdiseases

Biological Info:
http://usamrid/detrick.army.mil

Bioterrorism Readiness Plan: A Template for Healthcare Facilities
http://www.cdc.gov/ncidod/hip/Bio/13apr99APIC-CDCBioterrorism.PDF

Blue Book 4th Edition
http://usamrid.detrick.army.mil/education/bluebook.html

Chemical Agents of Warfare
http://www.cbwinfo.com/intro.html

Chemical Info:
http://ccc.apgea.army.mil

Chemical, Radiological & Nuclear Warfare Fundamentals
http://members.tripod.com/%7EMotomom/112CBRC

Emergency Repose to Terrorism:
http://www.usfa.fema.gov/downloads/pdf/ertss.pdf

HHS Select Biological Agents & Toxins

www.cdc.gov/od/sap

History of Bioterrorism
http://www.bioterry.com/HistoryBioTerr.html