

Office of Laboratory Services

Threat Preparedness Laboratory

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Today's Overview

- The Laboratory Response Network
- Your Laboratory Emergency Response Plan
- The Agents of Bioterrorism

The Laboratory Response Network (LRN)

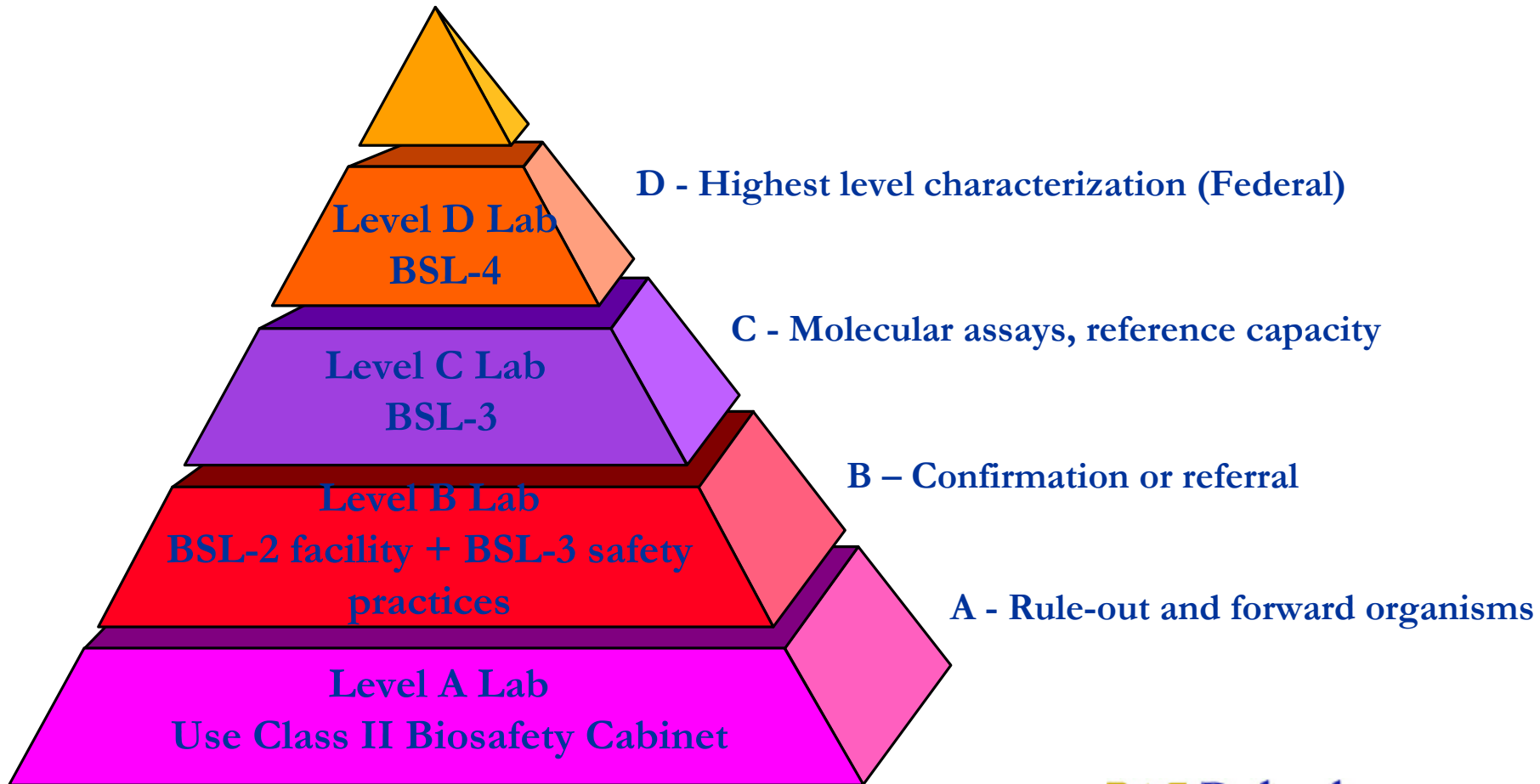
The Laboratory Response Network

- Created as part of the government bioterrorism response program
- Interconnected system of laboratories
- Provide standardized testing in response to a bioterrorism event

The Laboratory Response Network

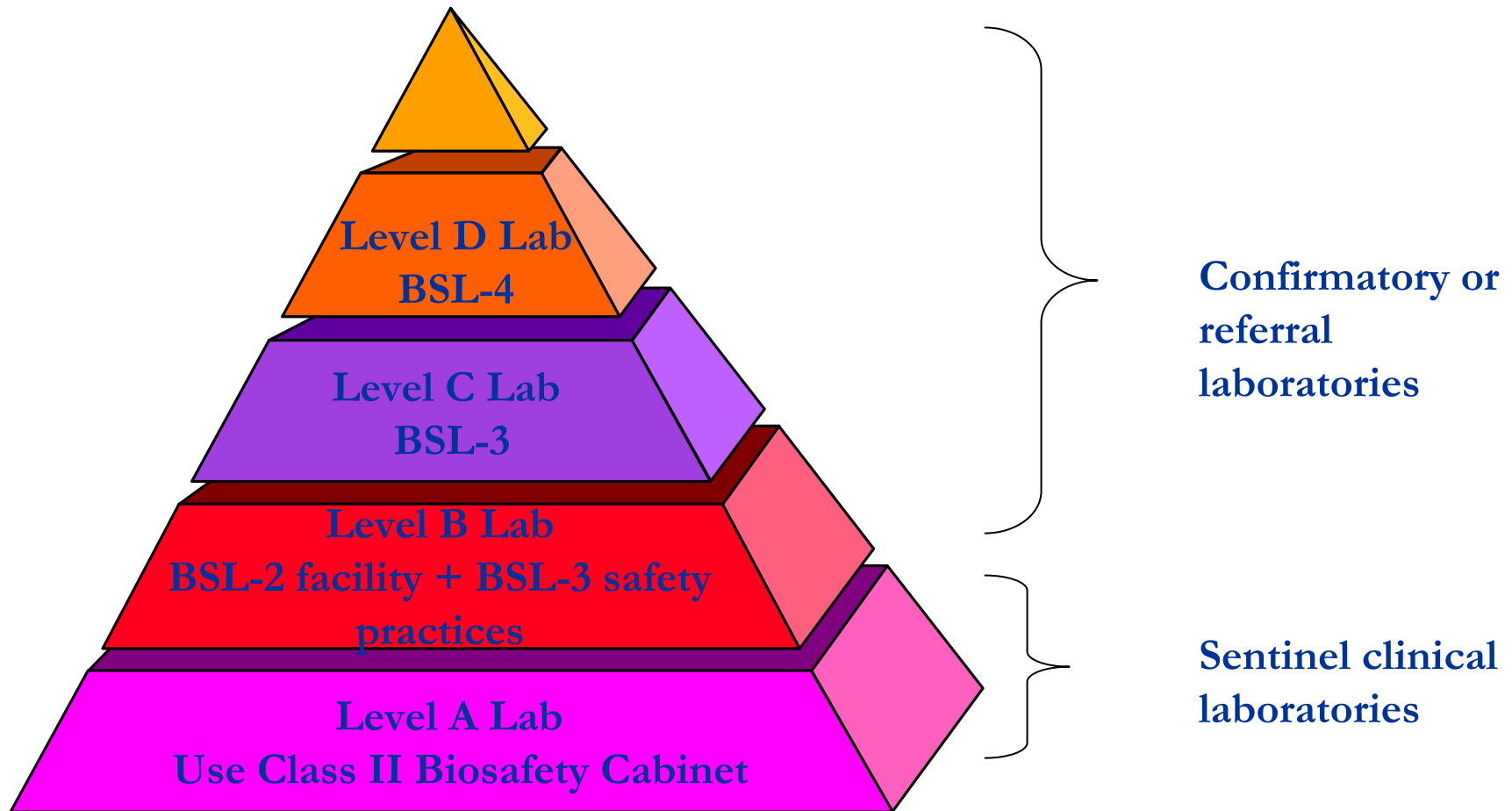
- Network initially included:
 - clinical, state public health, and selected federal and military laboratories
- Now expanded to include:
 - veterinary, food, environmental, chemical, and international laboratories

The Laboratory Response Network



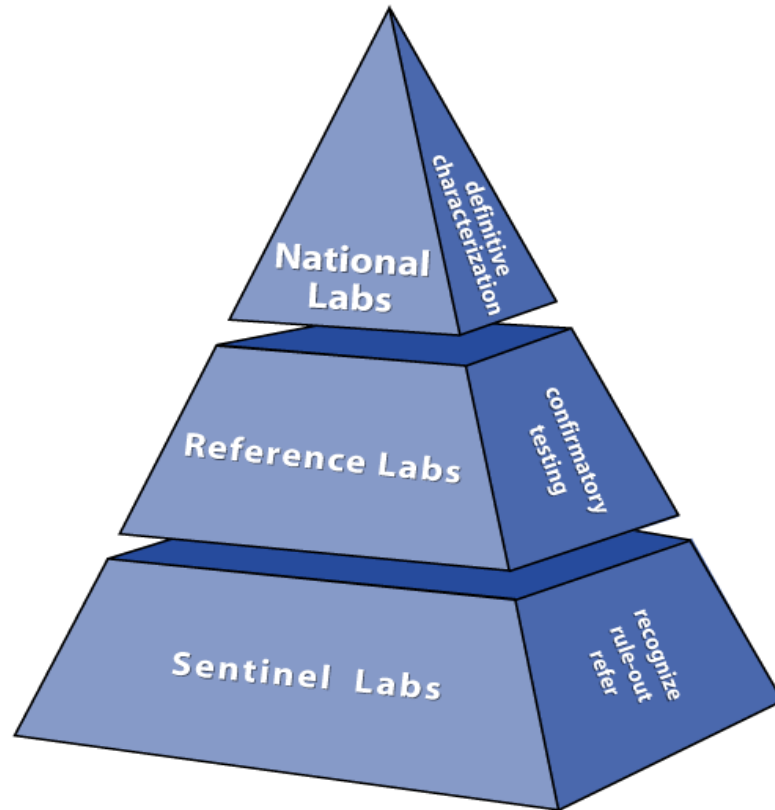
May 23rd & 24th, 2005

The Laboratory Response Network



May 23rd & 24th, 2005

LRN Today



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The LRN Sentinel Laboratory

The Level A laboratory is now the Sentinel
Laboratory

- Clinical laboratories doing microbiology and operating at BSL-2 (BMBL) would be “Sentinel Laboratory Capable”
- Ability to follow Sentinel Lab protocols
- Participation voluntary, unless your state laboratory director deems your lab as a Sentinel Laboratory

The LRN Reference Laboratory

Level B and C Laboratories are now LRN Reference Laboratories

- State Public Health Laboratories and selected large city or county laboratories
- Registered with the LRN and connected through a secure website for information, supplies, and reagents related to threat preparedness

The LRN National Reference Laboratory

Level D Laboratories are now the LRN National Reference Laboratories or Confirmatory Laboratories

- Have highest biosafety level to work with highly dangerous/infectious agents (smallpox, Ebola)
- They have methods to further characterize isolates

Your Laboratory Emergency Response Plan

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Your Laboratory Emergency Response Plan

- Develop and implement an all-hazards emergency response plan
- Train your staff and test your plan
- Incorporate Sentinel protocols into your routine SOPs
- Know whom to call, how and where to refer suspect isolates
- Provide a chain of custody or know where to find one
- Review and update as needed

Your Laboratory Emergency Response Plan

Be ready to help assess the threat:

- Number of infections
- Does it fit seasonal patterns
- Zoonoses/geography

Your Laboratory Emergency Response Plan

As a Sentinel Laboratory

- Be vigilant
- Have a terrorism response plan in place and practice it
- Be able to rule out threat agents

OR

- Refer to higher level laboratory

The Agents of Bioterrorism

Discussed today:

Bacillus anthracis

Yersinia pestis

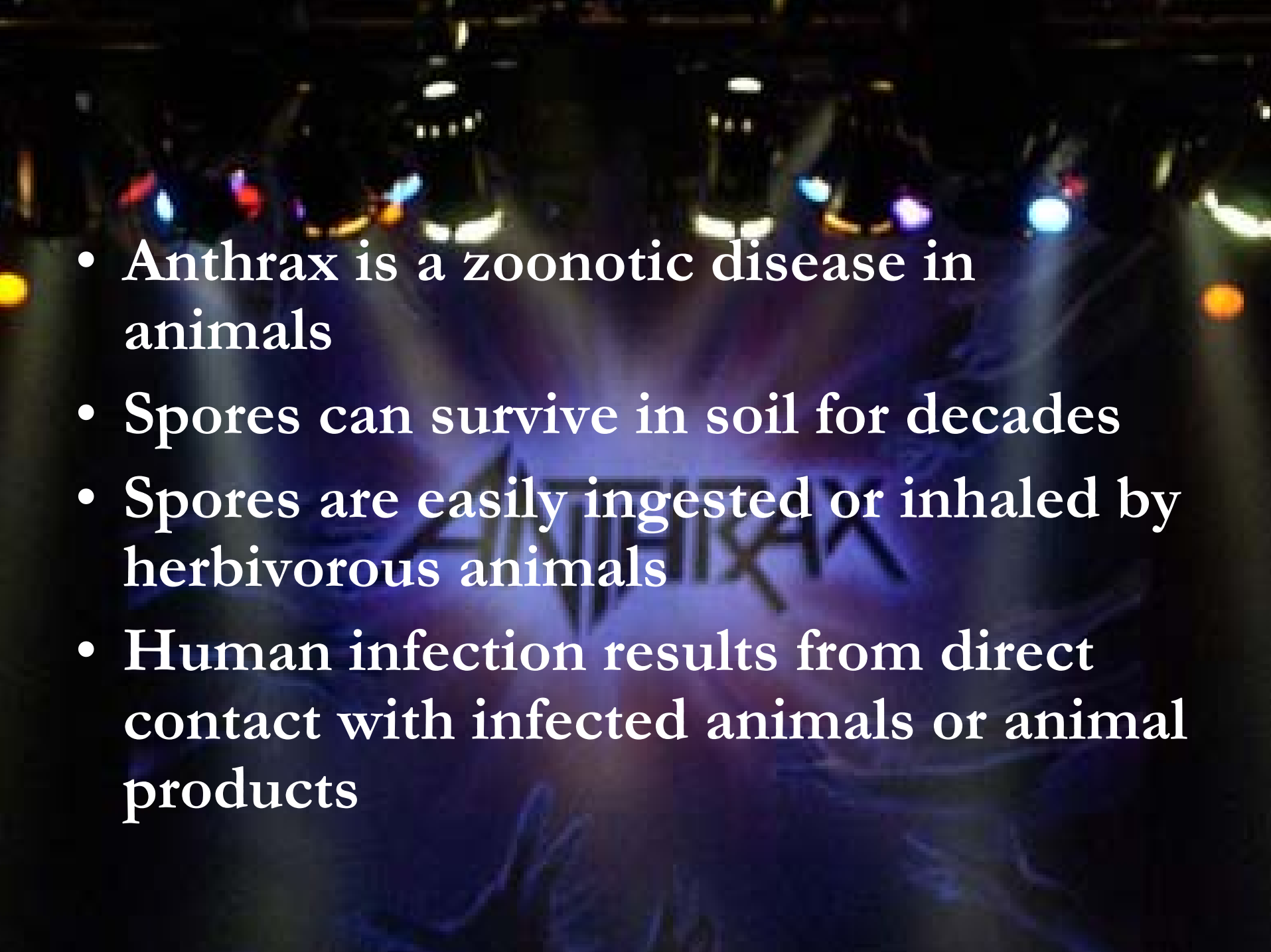
Brucella spp.

Francisella tularensis

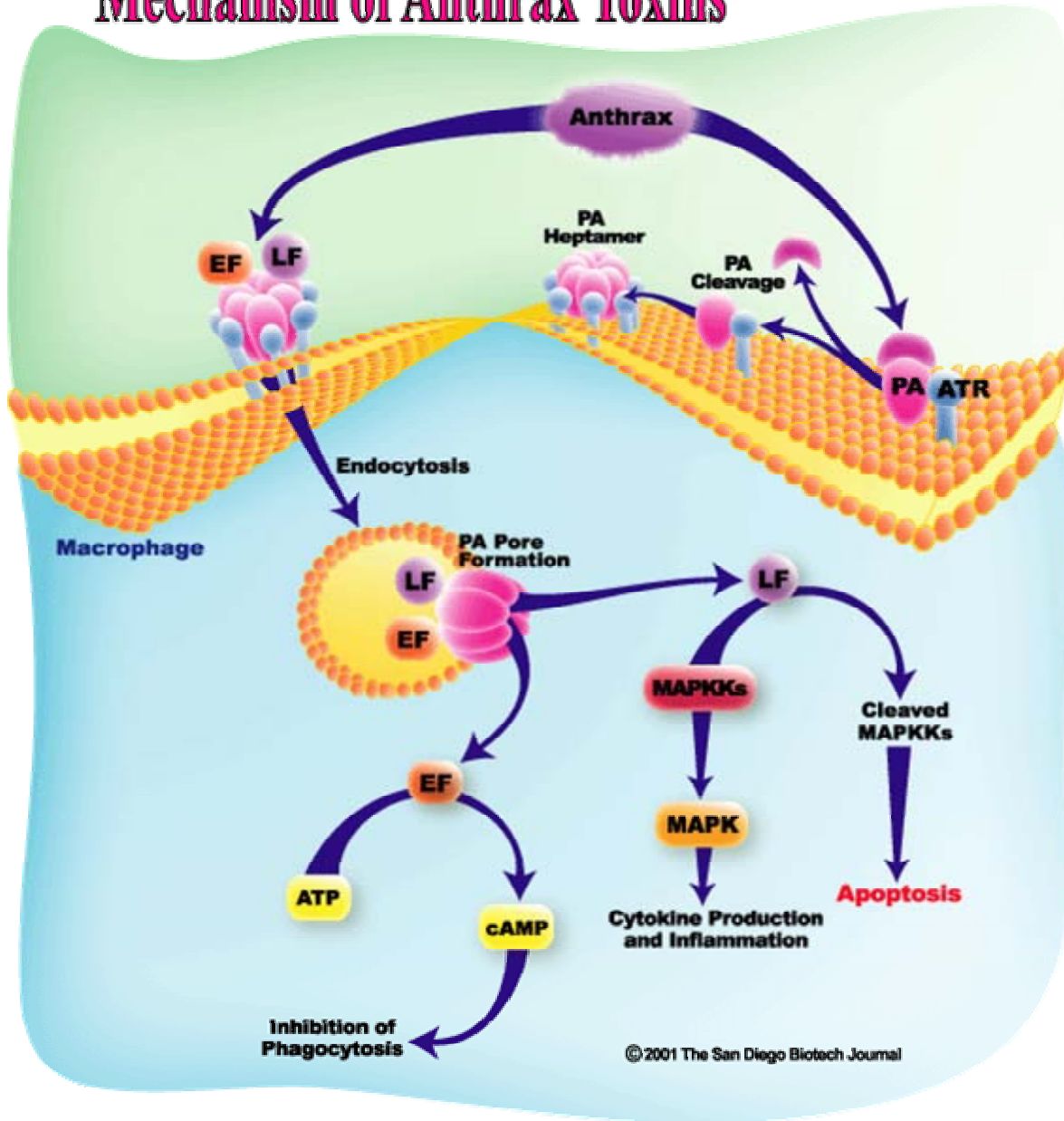
Botulism

Ricin Toxin

Pox Viruses

- 
- Anthrax is a zoonotic disease in animals
 - Spores can survive in soil for decades
 - Spores are easily ingested or inhaled by herbivorous animals
 - Human infection results from direct contact with infected animals or animal products

Mechanism of Anthrax Toxins



- ## Three Forms of Anthrax
- Cutaneous
 - Gastrointestinal
 - Inhalational

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Cutaneous Anthrax

- Spore enters skin
- Incubation of 2-3 days
- Papule develops
- Black eschar forms



“Anthrax” = coal (Greek)

- Vesicle or eschar should be lifted
 - fluid filled swab
 - aspirate from vesicle

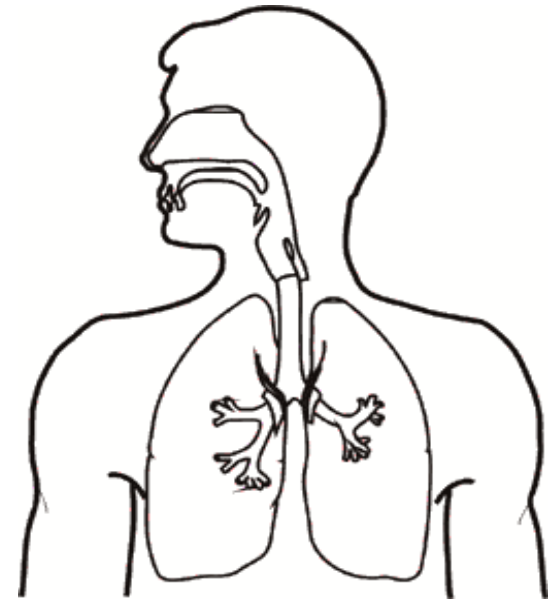
Gastrointestinal Anthrax

- Spore is ingested
- Incubation period of 2-3 days
- Acute inflammation of intestinal tract
- Vomiting of blood
- Severe bloody diarrhea
- Stool collection - preferably prior to antibiotics



Inhalational Anthrax

- Most deadly form
- Likely form in a bioterrorism event
- Spore is inhaled
- Incubation period of 1-6 days
- Spores are engulfed by macrophages
- Hemorrhagic mediastinitis
- Hemorrhagic lymphadenitis



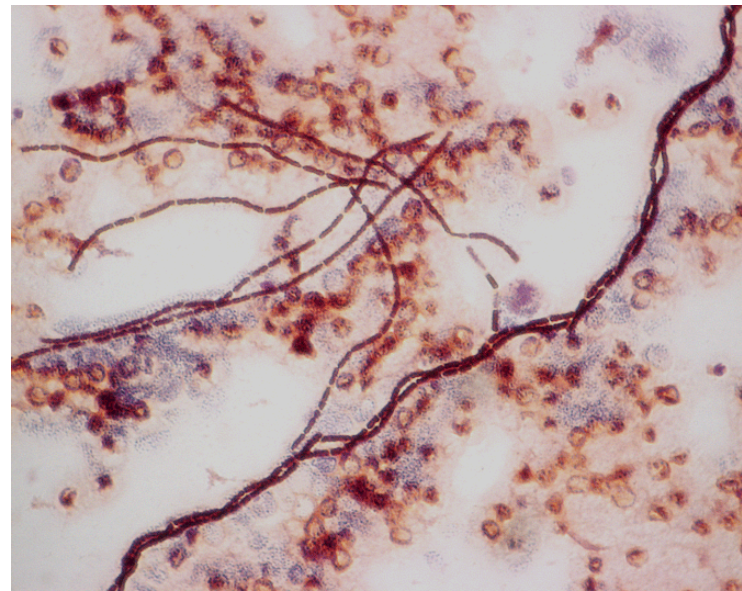
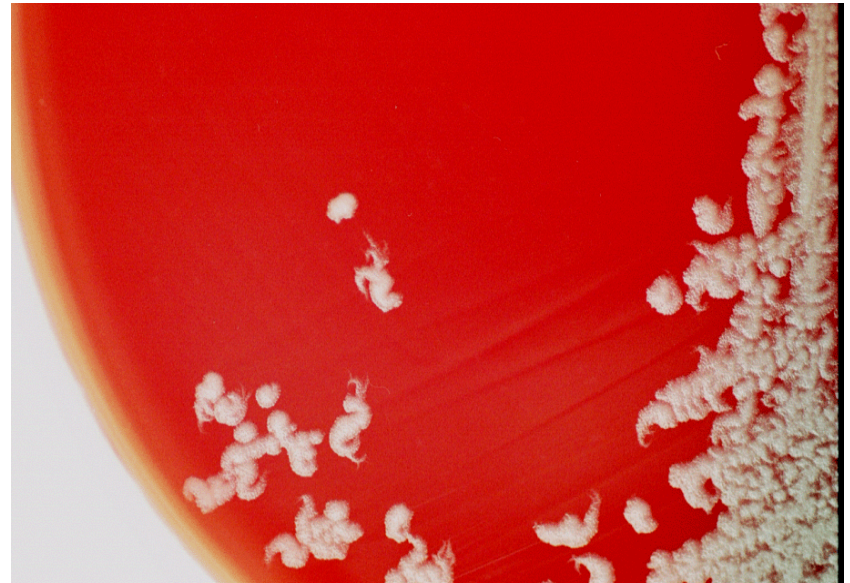
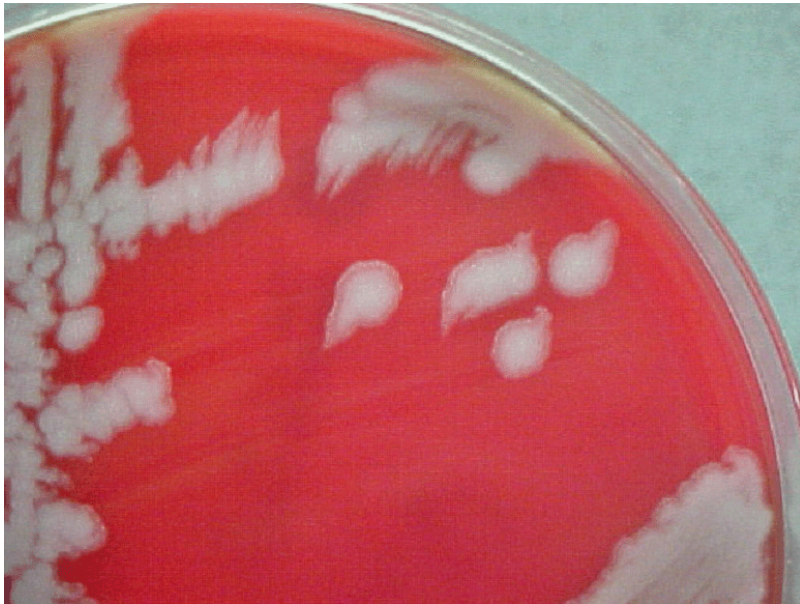
Sentinel Level Testing

Bacillus anthracis

Colony Morphology

- Rough, “ground glass” colony
- Irregular edges with “comma-like” projections
- Non-hemolytic
- “Sticky” , tenacious colony

24 hr. Growth of *B. anthracis* on SBA

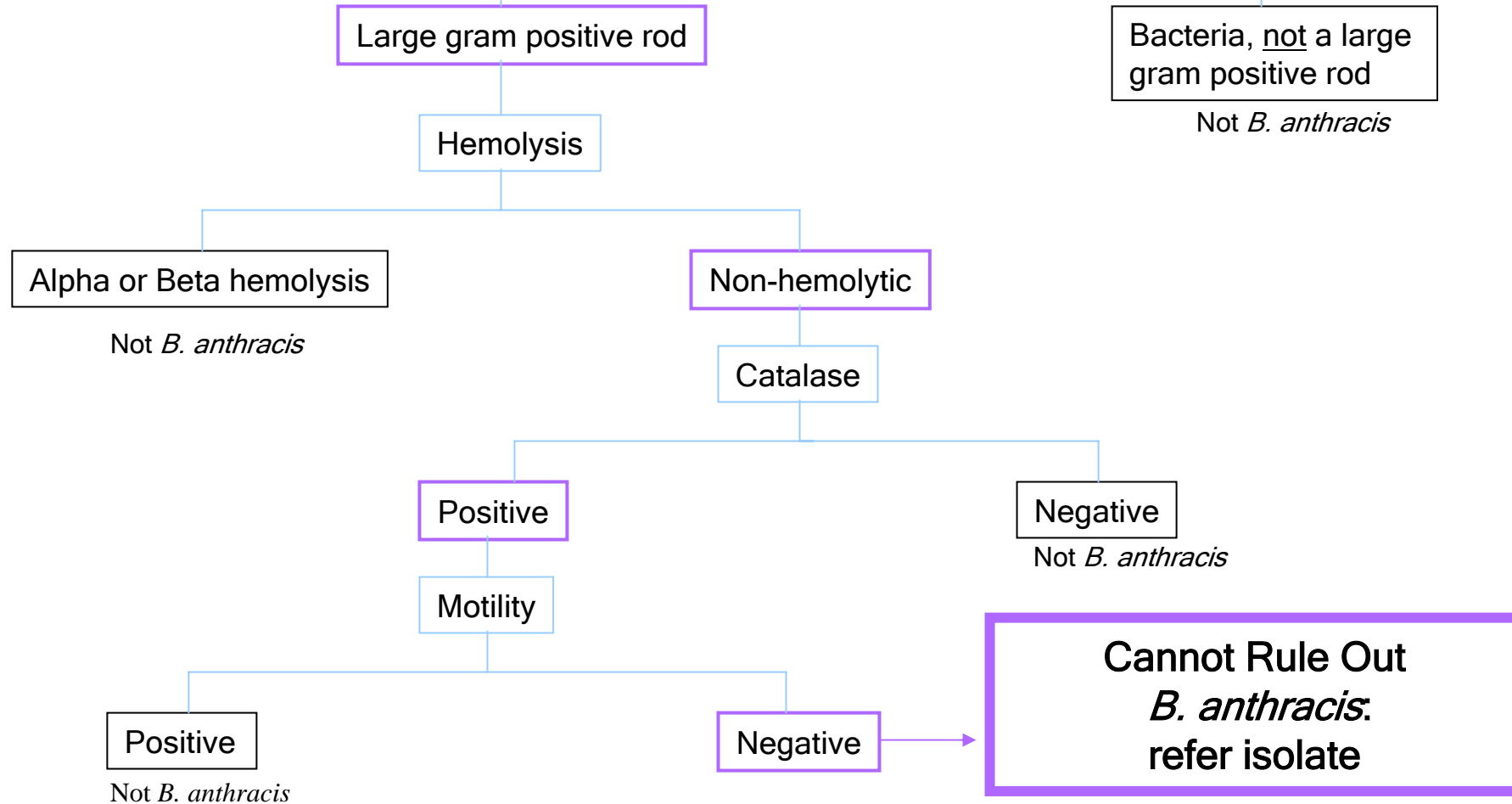


Colony Morphology

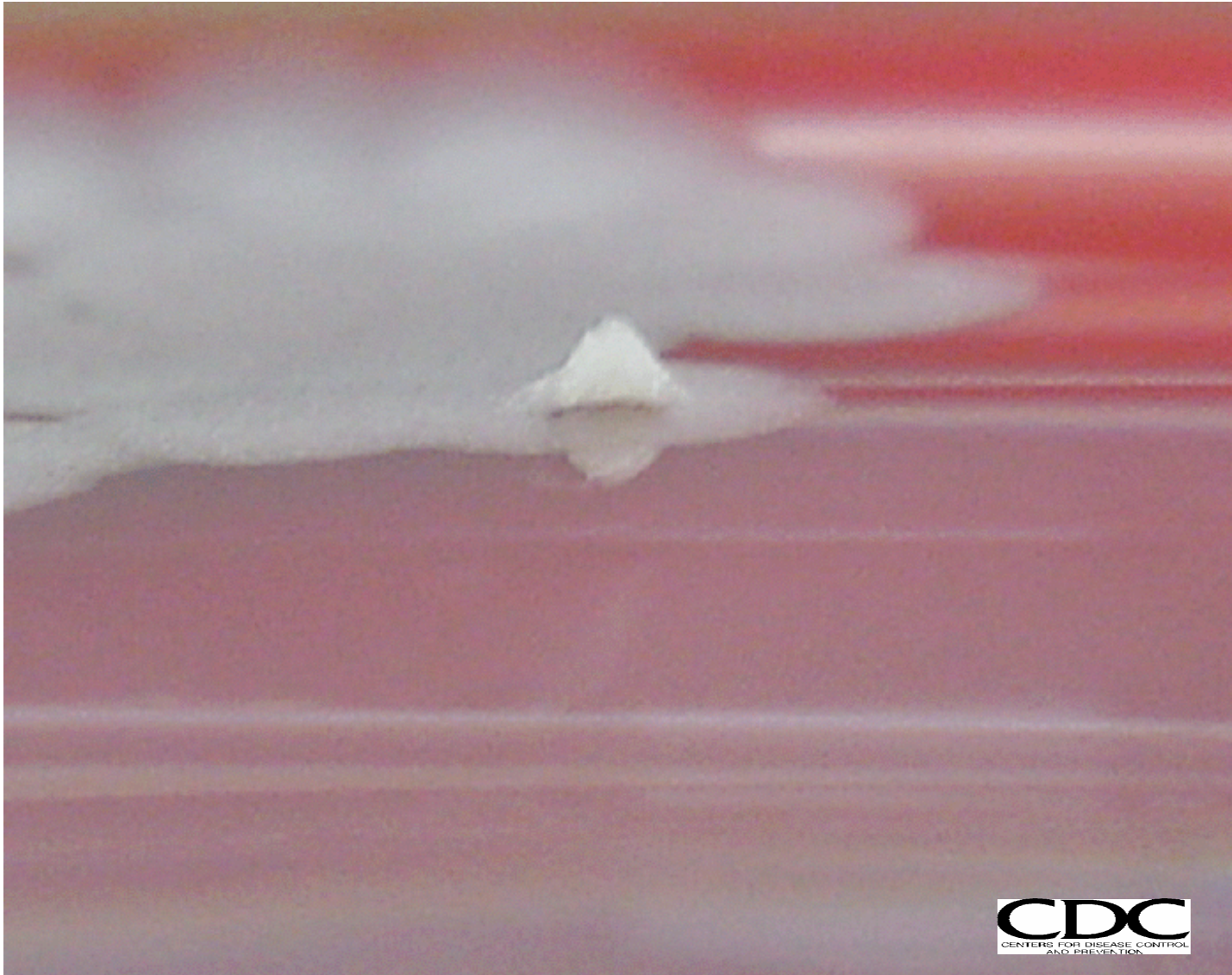
- Large, rough “ground glass” colony
- Irregular shaped edges, comma-like projections
- “sticky” - adheres to the agar
- Non- Hemolytic

Gram Stain Morphology

- Large gram positive bacilli
- 1-1.5 x 3-5 μm
- May have spores present



B. anthracis, tenacity of Colony



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Anthrax?

No, this isolate is hemolytic.



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Reference Level Identification

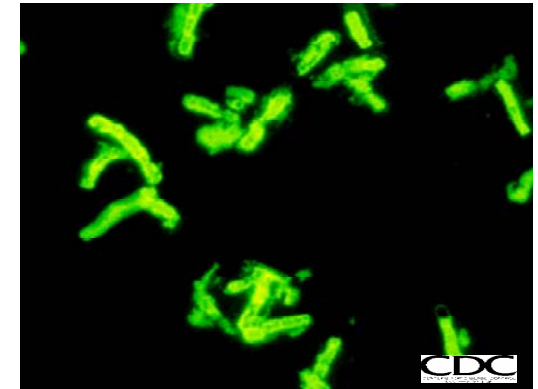
Bacillus anthracis



Phage Lysis



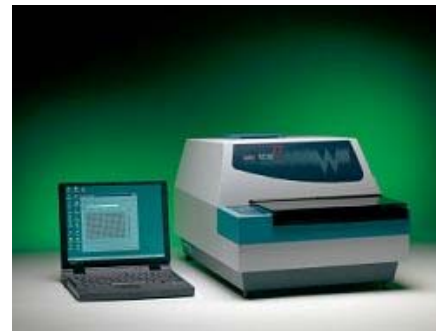
Visualization of
Capsule



DFA



Molecular
Methods



Time Resolved
Fluorescence



Yersinia pestis

Three plague pandemics



- First pandemic
 - antiqua ribotype O
- Second pandemic
 - mediaevalis ribotype O
- Third pandemic
 - orientalis ribotype B

Three Forms of Plague

Yersinia pestis

Bubonic

Most common, naturally occurring, form of the disease

Plague bacteria is transported to the lymph node closest to the flea bite site

Lymph node becomes inflamed and enlarged = “bubo”

Specimen Collection- aspirate from infected lymph node or “bubo”

Septicemic

Yersinia pestis infection in the blood

Specimen collection- Series of 3 venipuncture specimens taken 15-30 minutes apart

Pneumonic

1° pneumonic plague results from inhalation of plague infected aerosols 1) Infected person, 2) Infected animal, 3) Bioterrorism incident

2° pneumonic plague may result from bubonic plague

Sentinel Level Testing

Yersinia pestis

Colony Morphology

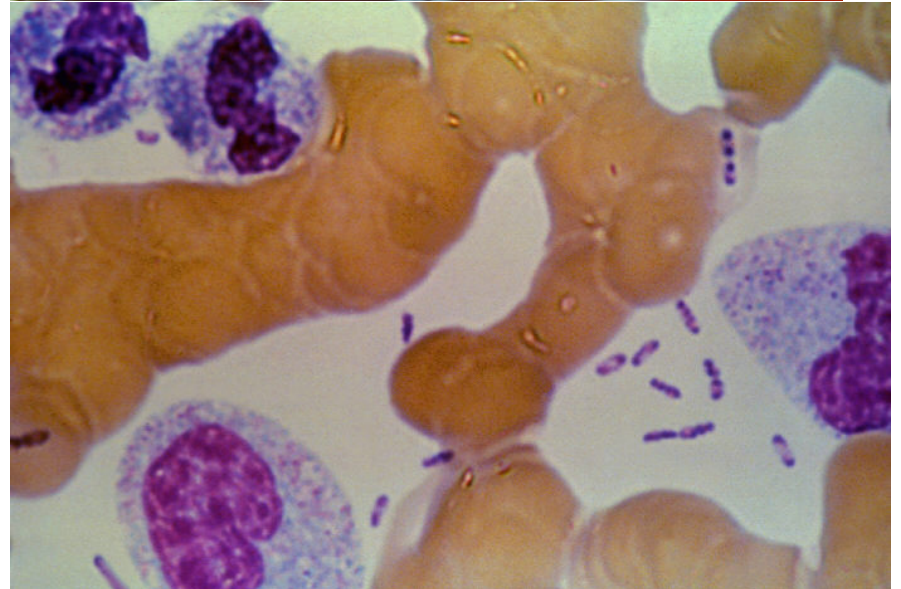
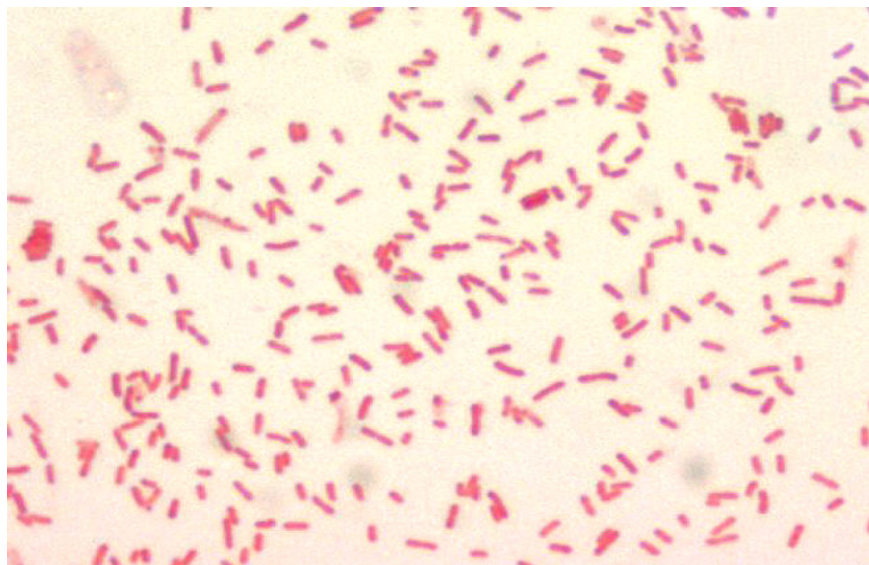
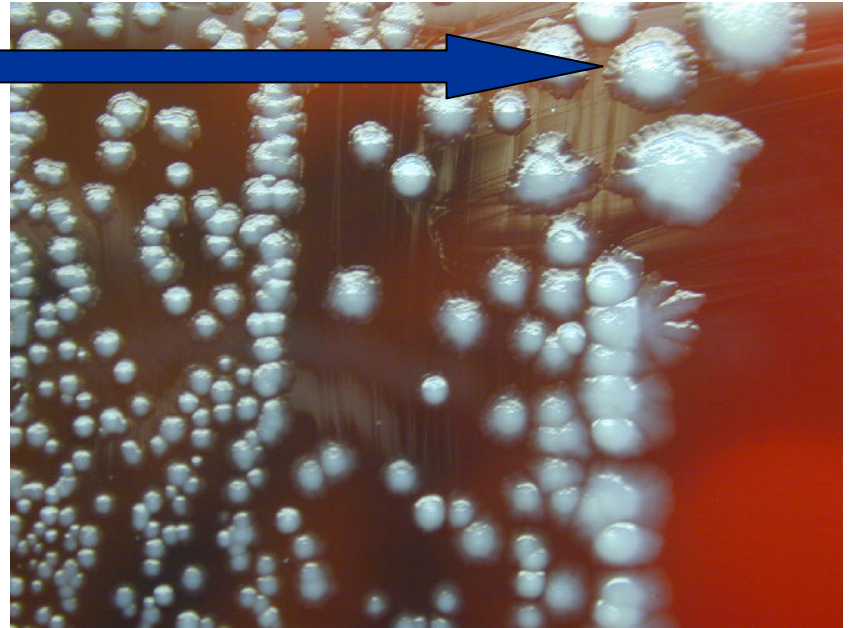
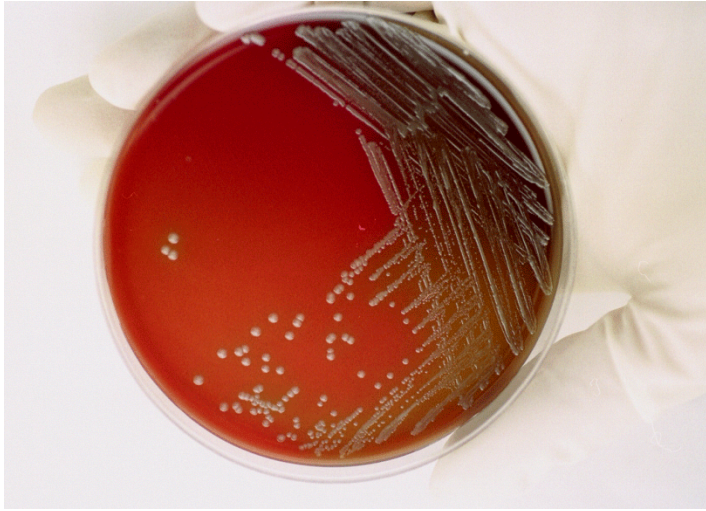
Pinpoint, gray-white, translucent colonies at 24 hours

At 48 hours colonies are non-hemolytic, resemble typical enteric gnb

After prolonged incubation, have “fried egg” appearance

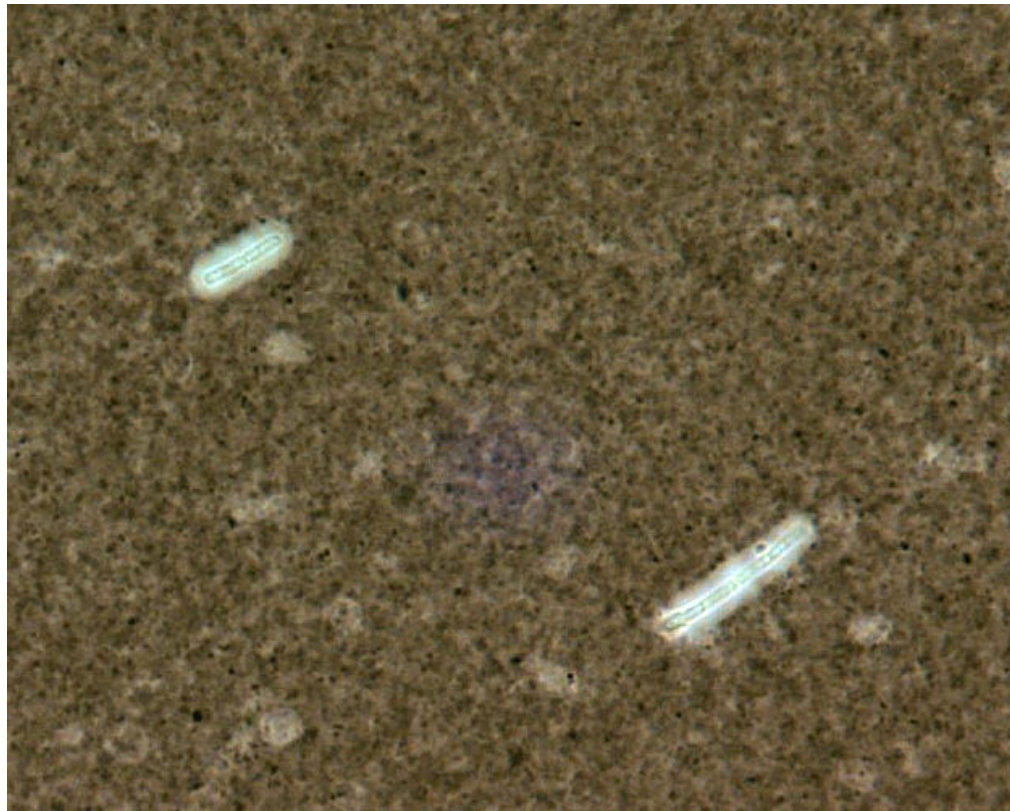
48 hour growth of *Y. pestis*

Fried egg appearance



Yersinia pestis

India Ink for F1 Antigen



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Sentinel Level Testing

Y. pestis

Gram Stain Morphology

Fat gram-negative bacilli, bipolar staining “safety pin”, 1.0 x 0.5 microns, single, pairs or short chains

Grows in/on:

Sheep blood agar (BA), Chocolate agar (CA), MacConkey (MAC) small Lac neg, Brain heart infusion broth (BHI), and most routine blood culture systems

Organism Characteristics:

Grows better at room temperature than most enteric gram negative rods, may cause problematic identification with automated methods, should be confirmed by classical biochemicals

Classical Biochemical Reactions

Oxidase: negative

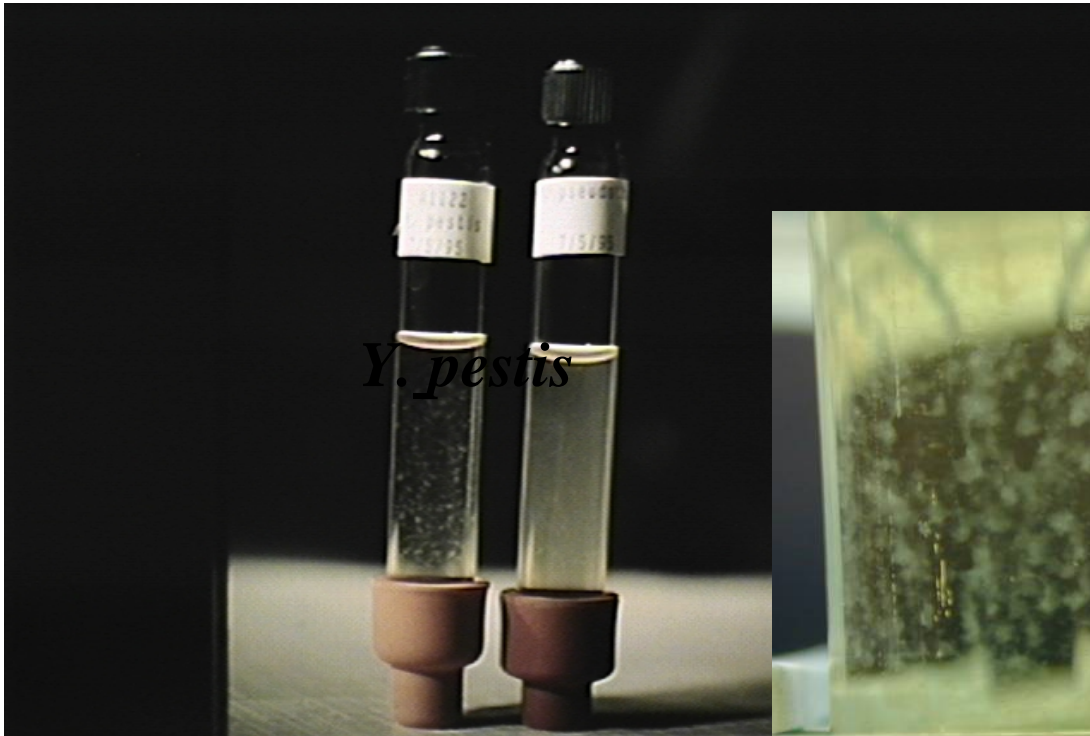
Urea: negative

Motility: Negative

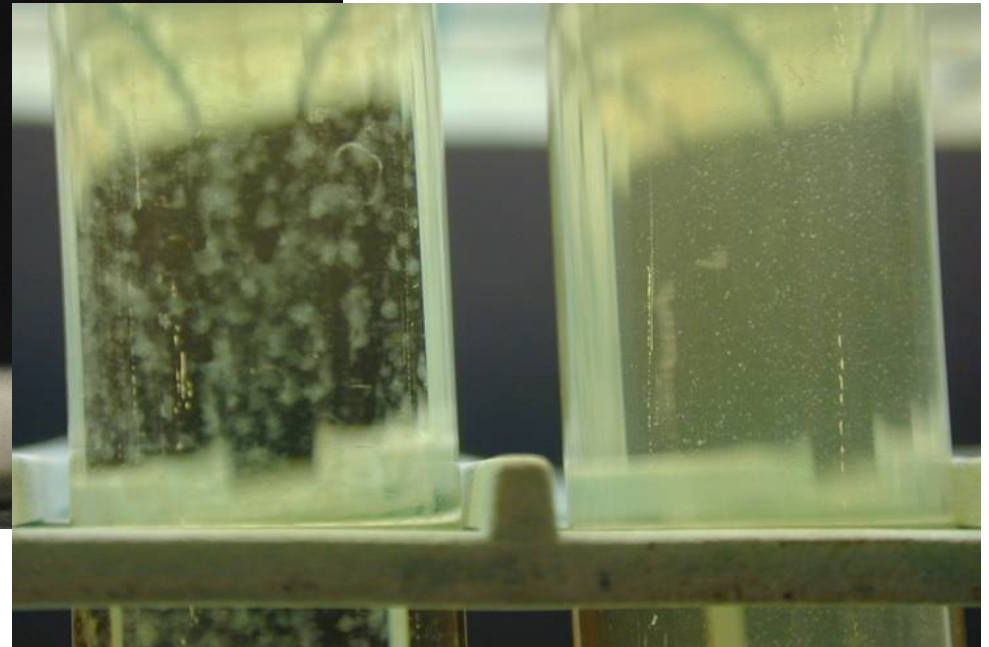
Sugar Fermentation: Only glucose and mannitol are positive

Growth in Broth

Yersinia pestis

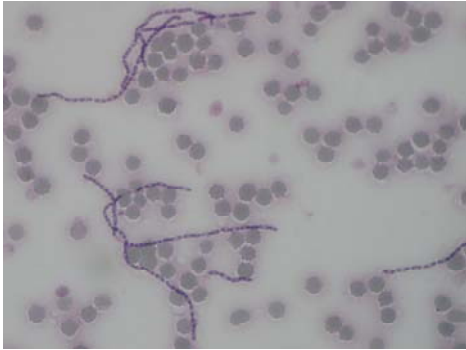


CDC Photo

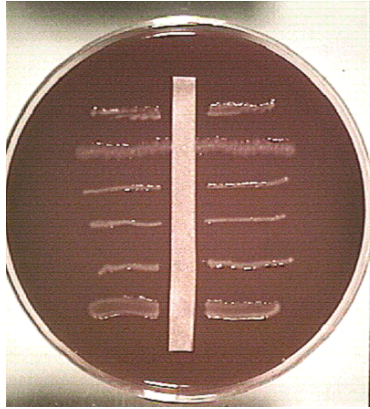


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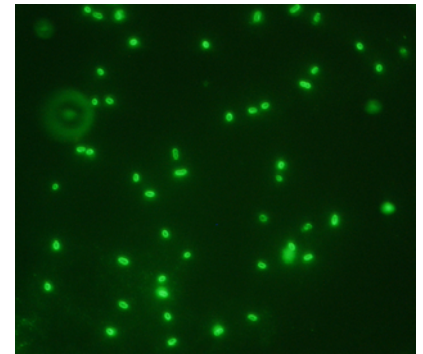
Reference Level Identification *Yersinia pestis*



**Giemsa Stain
(bipolar staining)**



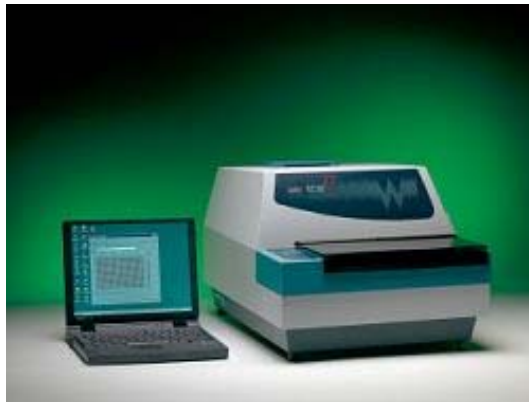
Phage Lysis



**DFA F1 antigen stain
of *Y. pestis* from
culture**



**Molecular
Methods**



**Time Resolved
Fluorescence**

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Brucellosis

Any of four *Brucella* spp. can cause human infection

- *B. melitensis* (from sheep & goats)
- *B. suis* (from pigs)
- *B. abortus* (from cows)
- *B. canis* (dogs—rarely causes disease)



Brucellosis

- Causes septicemic febrile illness in humans
 - Non-specific symptoms: irregular “undulating” fevers, headache, profound weakness and fatigue, chills/sweating
 - Focal lesions may occur in bones, joints, genitourinary tract, and other sites
- Incubation period is highly variable, ranging from 5 days to 6 months, relapses are common
- Low mortality rate (<5%), but can be extremely incapacitating
- *B. melitensis* and *B. suis* most pathogenic for man, *B. canis* and *B. abortus* less so
- Aerosols are highly infectious
 - Infective dose = 10 -100 organisms

Sentinel Level Testing

Brucella spp.

Colony Morphology

- 48 hour growth colonies are small, raised, glistening, white-cream, non-hemolytic, may not be visible at 24 hours

Gram stain Morphology

- Very tiny: 0.5-0.7 x 0.6 microns, faintly staining, Gram-negative coccobacilli “Fine sand”

Grows in/on:

- Sheep blood agar (BA), Chocolate agar (CA), Thayer-Martin (TM), selective medium, MacConkey (MAC) negative or poor growth, Brain heart infusion broth (BHI)

Automated identification: Most likely will not identify this organism

Classical biochemicals:

Oxidase – positive

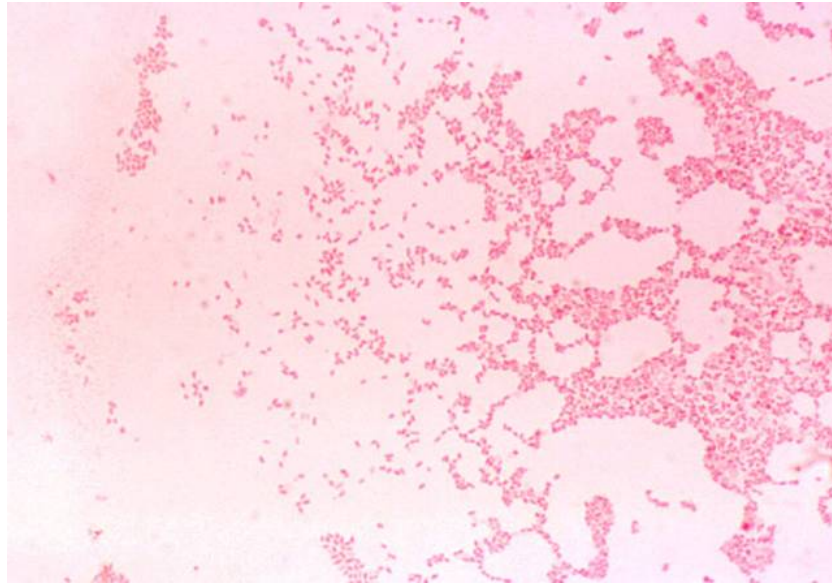
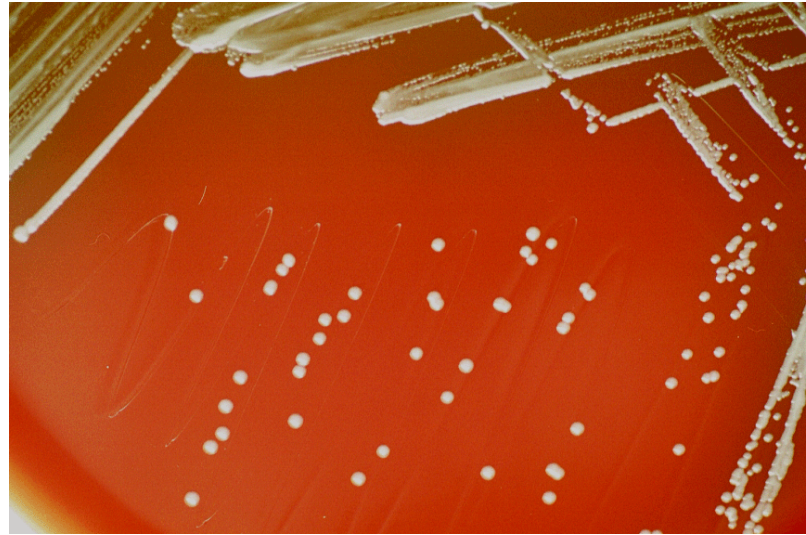
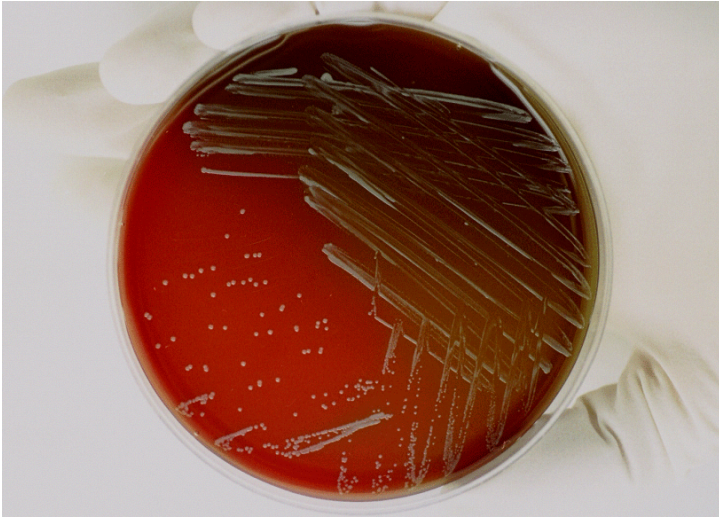
Urea – positive ...sometimes rapid** (*B. canis*, *B. suis* 15min, *B. abortus*, *B. melitensis* 24 hrs)

Nitrate - positive

Motility – negative

Does *not* need X or V factor

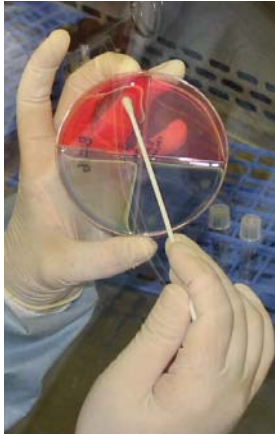
72 hour growth of *B. abortus* on SBA



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Reference Level Identification

Brucella spp.



Dye Tolerance
Test

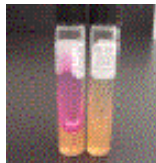
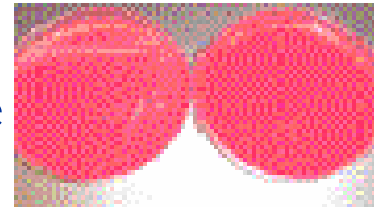


H₂S
Production



Slide agglutination

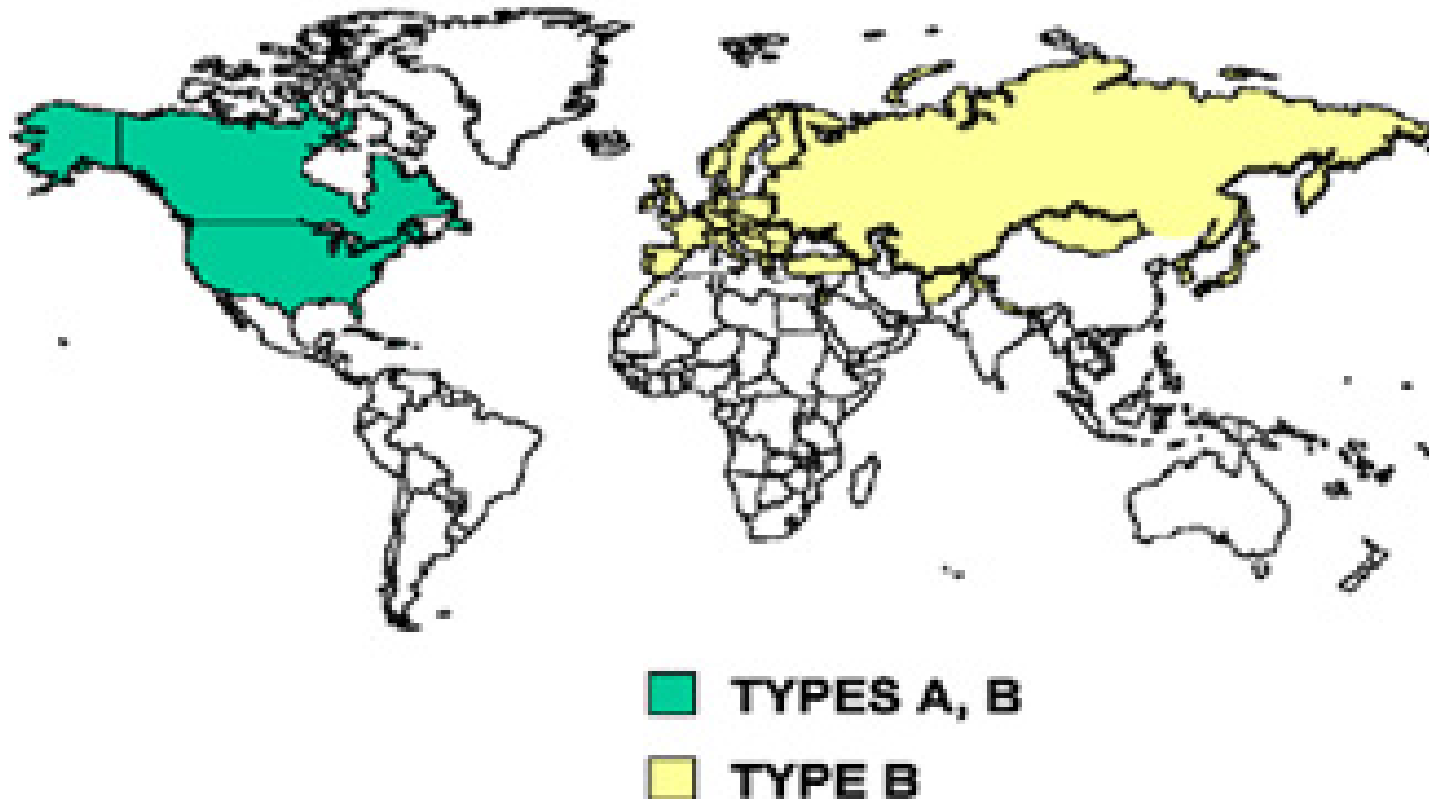
CO₂ Dependence



Urease—Time to positive

Francisella tularensis

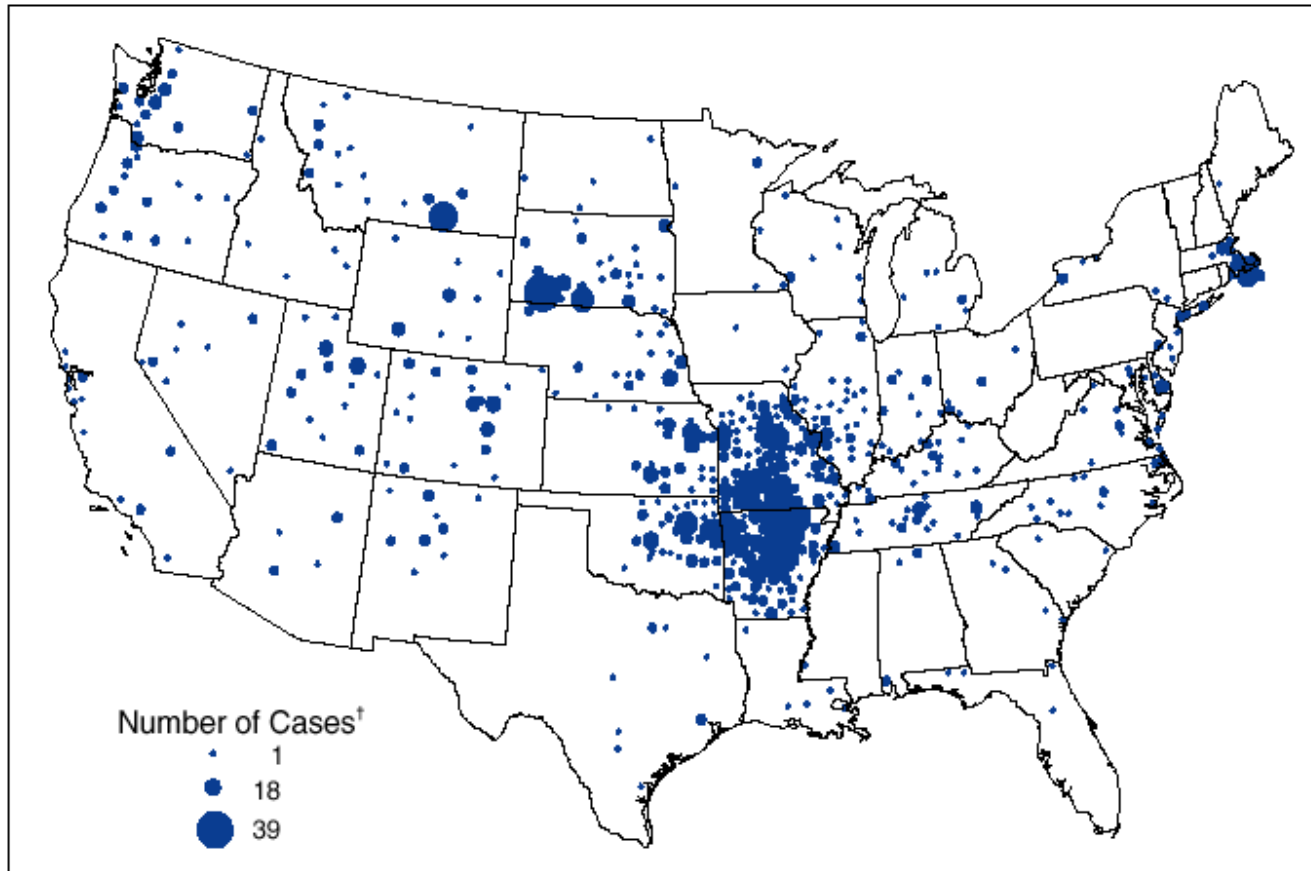
GEOGRAPHIC DISTRIBUTION OF TULAREMIA



Tularemia

Approximately 200 cases per year in U.S.

FIGURE 2. Reported cases* of tularemia — United States, 1990–2000



Francisella tularensis

Biotypes:

- *Francisella tularensis* subsp. *tularensis*
- *Francisella tularensis* subsp. *holarctica*
- *Francisella tularensis* subsp. *mediaasiatica*
- *Francisella novicida*

Taxonomy:

- *Francisella tularensis* and *Francisella novicida* have a 99.6% 16S rDNA similarity
- *Francisella novicida* are sucrose negative and lack virulence for humans and rabbits
- *Francisella philomiragia* are oxidase positive (Kovacs modification), hydrolyze gelatin, low virulence

Types of Tularemia

Ulceroglandular

Nonhealing ulcers on extremities, lymphadenopathy

Oculoglandular

Painful, purulent conjunctivitis, lymphadenopathy

Glandular

Like ulceroglandular form, but no ulcer

Oropharyngeal

Sore throat which is painful beyond its appearance & does not respond to penicillin

Pneumonic

Dry, nonproductive cough, dyspnea, fever

Gastrointestinal

Can range from mild, unexplained persistent diarrhea to bowel ulceration and death

Typhoidal (Systemic)

Bacteremia, delirium, prostration, and shock



Sentinel Level Testing

Francisella tularensis

Colony morphology

- Usually too small to see at 24 hours
- Chocolate Agar: 48 hours; colonies will be shiny, gray and flat, with smooth, entire edges.
- Cysteine Heart Agar (or BCYE): 48 hours; colonies will be greenish-white with a buttery consistency

Grows in/on:

- Thioglycollate broth, Cysteine-heart agar w/9% sRBCs, Thayer-Martin agar (TM) / GC-Lect, IsoVitaleX-supplemented agar, Chocolate agar (CA), Buffered charcoal yeast extract (BCYE), Grows very slowly in routine blood culture

Gram Stain Morphology

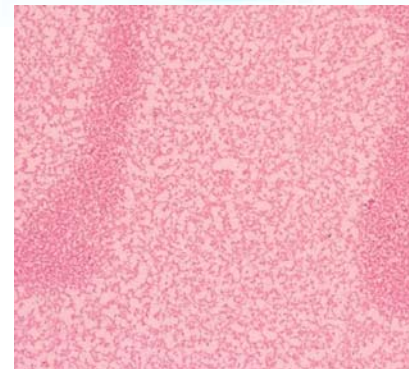
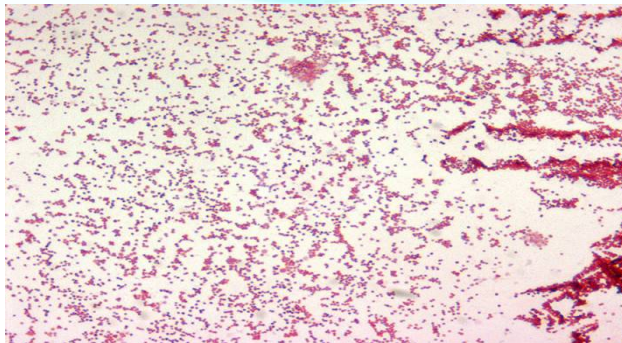
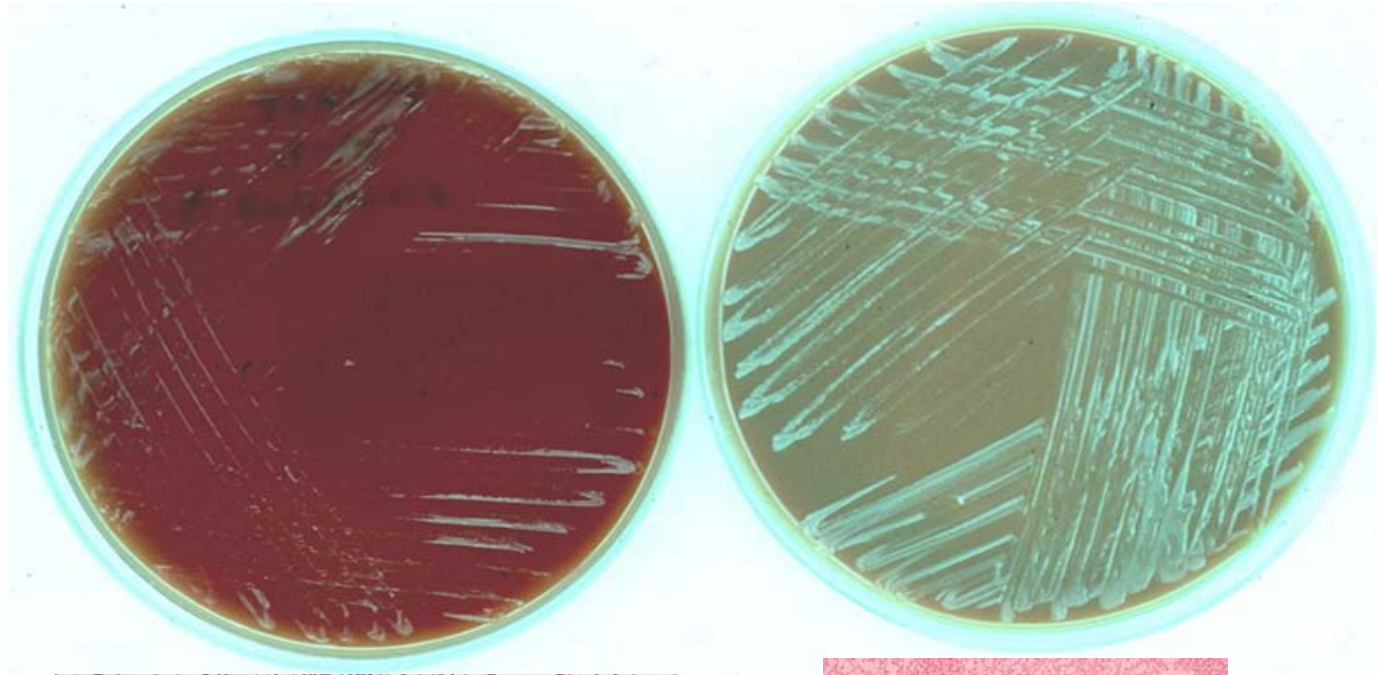
- Extremely tiny: 0.2-0.7 x 0.2 microns, poorly staining, pleomorphic, gram-negative coccobacillus

Automated identification: Most likely will not identify this organism

Classical biochemicals:

Oxidase – negative	beta lactamase – positive
Motility – negative	Does not require X or V
Urea – negative	

F. tularensis
on **blood** and **chocolate** agar

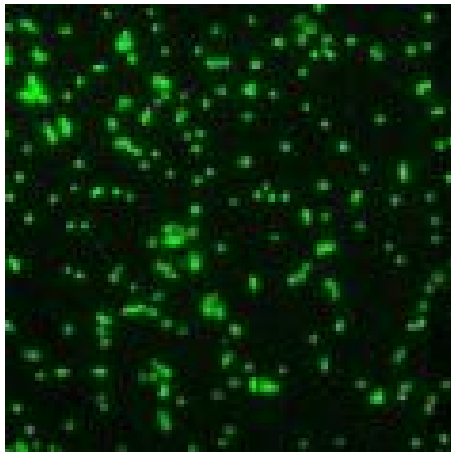


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Reference Level Identification

F. tularensis

DFA – cellular surface antigens



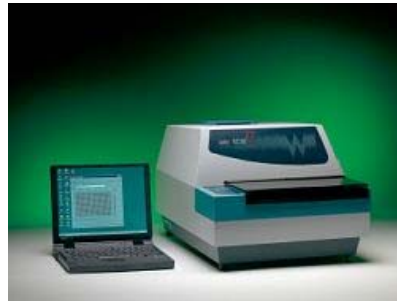
Classical
biochemicals



Molecular
Testing

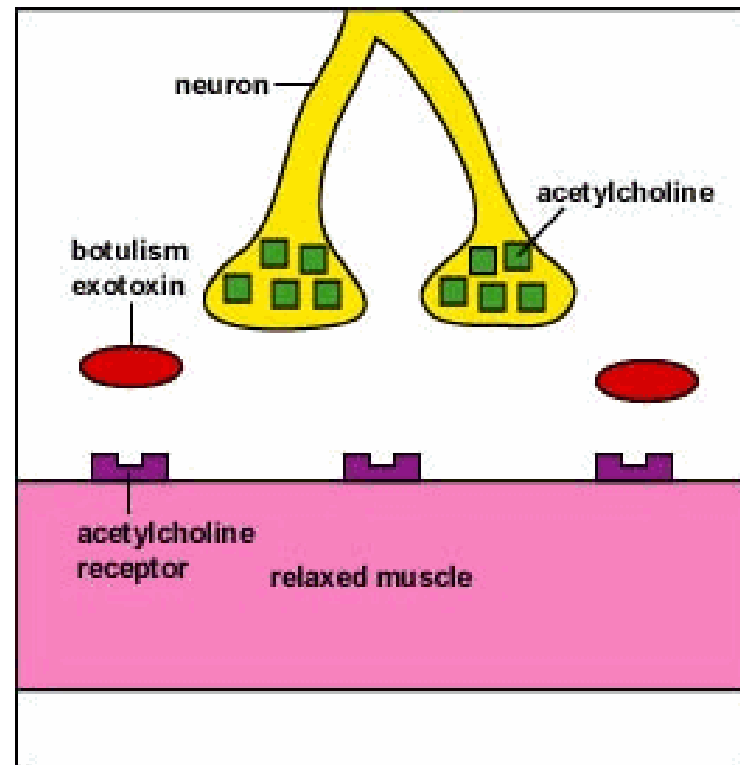


TRF



Botulinum neurotoxin producing species of *Clostridium*

- *Clostridium botulinum*
- *C. baratii*
- *C. butyricum*

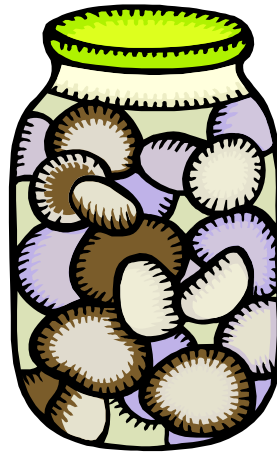


Botulism Types

Intentional Contamination



Foodborne



Infant (& adult colonization)



Wound



Sentinel Level Testing botulinum toxin

- There are no sentinel level tests for botulism toxin
- The diagnosis of botulism is made clinically, i.e., based on the patient's case history and physical findings
- Health care providers suspecting botulism should contact the state public health laboratory or state reference laboratory **IMMEDIATELY!**

Suggested Specimens Based on the Form of Botulism Presented

Foodborne

- Clinical material: Serum, gastric contents, vomitus, stool, return from sterile water or saline enema
- Autopsy samples: Intestinal contents and gastric contents (serum if available)
- Food samples

Infant

- Feces
- Return from sterile water or saline enema
- Serum: Although circulating toxin may be detected in infants with botulism, it is rare. Shipment of other specimens should not be delayed while waiting for serum collection.
- Postmortem samples: Intestinal contents from different levels of small and large intestine
- Food and environmental samples as appropriate for the investigation

Wound

- Serum
- Exudate, tissue, or swab samples of wound transported in an anaerobic transport medium
- Feces or return from sterile water enema (wound may not be source)
- An isolate of suspected *C. botulinum* (maintain under anaerobic conditions)

Intentional toxin release (inhalational or ingested)

- Serum
- Feces or return from sterile water enema
- Food, solid or liquid
- Environmental or nasal swabs
- Gastric aspirate

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Laboratory Capacity for Botulinum Toxin Testing



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Botulism Reference Level Laboratory Diagnosis

- Mouse bioassay is the only currently approved test for the laboratory confirmation of botulism
- An ELISA (FDA/CDC) was recently validated for toxin detection in culture





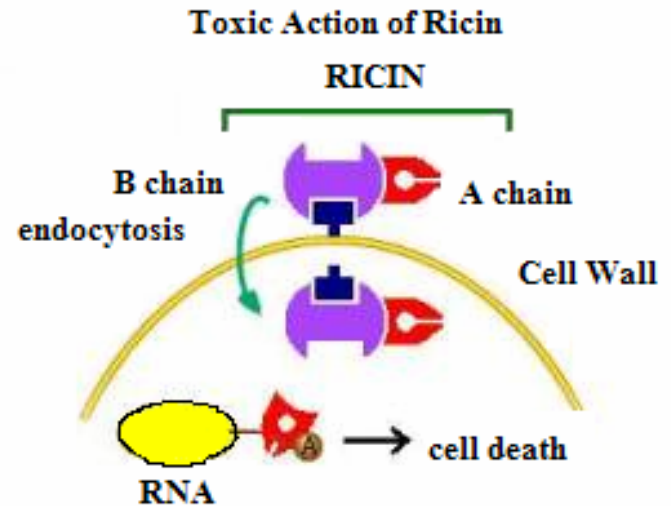
Ricin Toxin



- February 4, 2004, the Secret Service acknowledged that ricin had been found at a White House mail-processing center in early November 2003
- Ricin discovered at a U.S. Postal facility in Greenville, SC on October 15, 2003
- September 7th 1978, Georgi Markov, a Bulgarian exile journalist and writer, was jabbed in the thigh with an umbrella as he mounted the steps at the BBC in London.

Ricin Toxin

- Is a toxin extracted from a castor bean from the plant, *Ricinus communis*
- Ricin does not partition into castor oil because it is water-soluble
- Ricin toxin contains two parts, Ricin and RCA (hemagglutinin)
- Ricin contains an A chain, ribosome inactivating protein, and a B chain, cell binder
- 1,000 X **less** toxic than Botulinum toxin



Ricin Poisoning

Gastrointestinal or Inhalational



- Castor bean is eaten or toxin inhaled
- Symptoms occur within hours
- Respiratory distress within 8 hours after exposure
- 1 mg can kill an adult
- 1 castor bean can kill an infant

Sentinel Level Testing

Ricin Toxin

- There are no sentinel level tests for ricin toxin
- The diagnosis of ricin poisoning is made clinically, i.e., based on the patient's case history and physical findings
- Health care providers should contact the state public health laboratory or state reference laboratory **IMMEDIATELY!**

Ricin Toxin Reference Level Laboratory Diagnosis

**Molecular Toxin
Testing for castor
bean DNA**



**Fluorescent
Antibody Assay on
biopsy tissue**

TRF



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Small Pox

Ramses V Mummy



Aztec Drawing



Pox Viruses

Orthopoxviridae family (dsDNA)

2 strains of Variola

Variola major

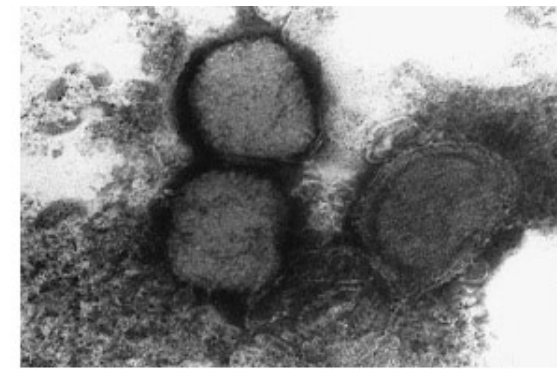
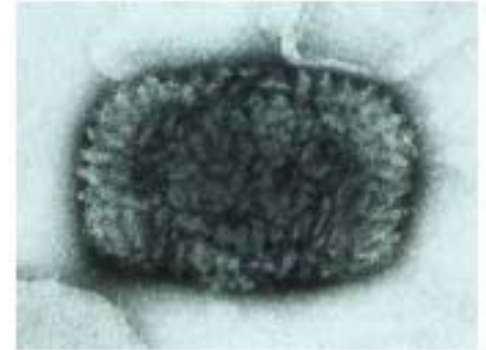
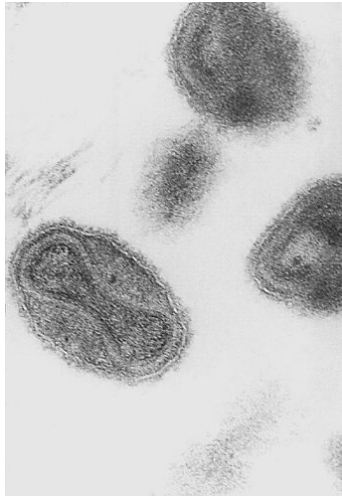
Variola minor

Vaccinia

Used for current vaccine

Cowpox or Catpox– used by Jenner in first vaccine

Monkeypox – rare but serious disease from monkeys in Africa



Variola (smallpox) as viewed through a microscope.

Stages of Variola Virus (Small Pox)

Incubation (7-17 days, not contagious)

Asymptomatic

Prodromal (2-4 days, sometimes contagious)

Nonspecific febrile illness, flu-like

Eruptive (contagious)

Characteristic rash, first on the buccal and pharyngeal mucosa, then on the face, forearms, and hands. Within a day, the trunk and lower limbs are involved; the back is more involved than the abdomen.

Distribution of the rash is centrifugal; profuse on the face, more abundant on the forearms than the upper arms, and on the lower legs than the thighs.

Small Pox

Four types of Variola Major pox:

1) Ordinary

Most frequent

2) Modified

Mild

Occuring in previously vaccinated persons

3) Flat (Malignant)

4) Hemorrhagic

Much shorter incubation

Not likely to be recognized as smallpox initially

Progression of Ordinary Small Pox Rash



Day 2



Day 4



Day 7



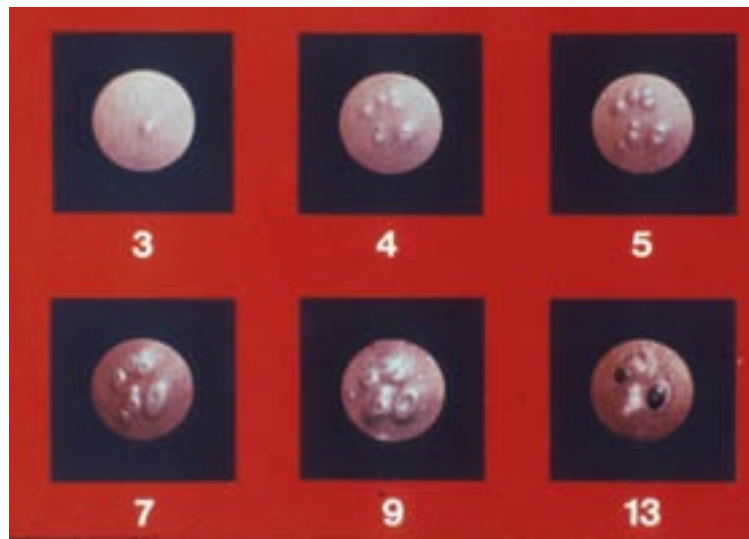
Day 8



Day 10



Day 20



May 23rd & 24th, 2005

Differential Diagnosis

Chickenpox (varicella)

Vesicles shallow, in crops,
varied stages

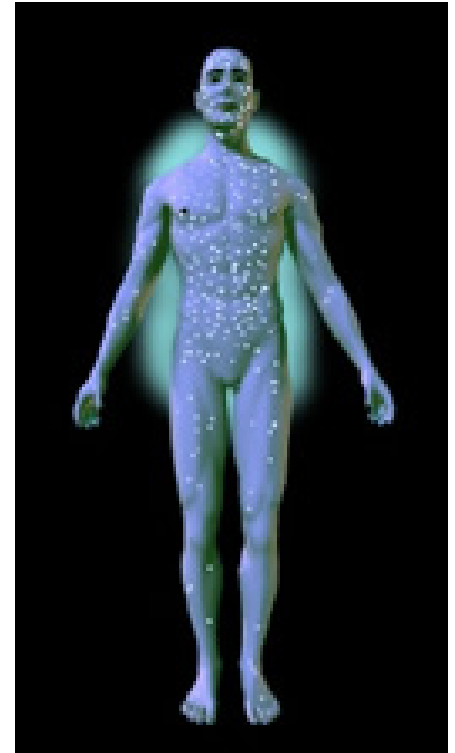
Axial or centripetal, spares
palms/soles

Other orthopox viruses

Monkeypox – only in Africa,
monkey contact

Vaccinia – after exposure to
vaccine

Cowpox – rare, only in UK



Sentinel Level Testing Variola Major (Small Pox)

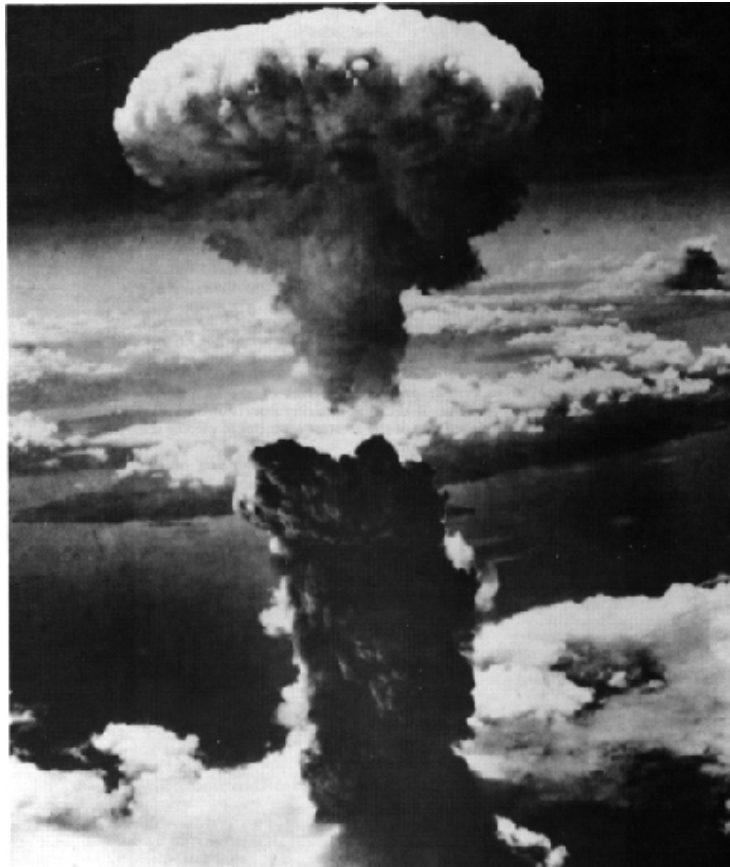
- There are no Sentinel level laboratory test for the identification of Variola virus.
- Immediately contact your state public health laboratory director and the state public health department epidemiologist. The state public health laboratory should immediately contact CDC at one of the following phone numbers:

CDC Emergency (Duty Officer), 24 hours a day, 7 days a week:
(770) 488- 7100

Rapid Response and Advanced Technology (RRAT) Laboratory:
(404) 639- 0075, (404) 405-7477, or (888) 374-1764



THE END



May 23rd & 24th, 2005