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# Theobald Smith Society

New Jersey Branch, American Society for Microbiology

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3/18/04

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Theobald Smith Society

## Joint Meeting with NJSIM

Speaker: Dr. Sherman Hom

NJ Department of Health, Div. Of Public Health Lab

Wednesday, March 17, 2004 Crowne Plaza Hotel

### "NEW JERSEY PUBLIC HEALTH LABORATORY BIOTERRORISM RESPONSE ACTIVITIES"

Hospitality: 5:30 pm

Lecture: 6:00 pm

Dinner: 7:15 pm

TSS

Dr. Hom is the director Bioterrorism Lab & Molecular Detection Services with the NJ Department of Health and Senior Services; Division of Public Health and Environmental Laboratories, located in Trenton, NJ. As the Director of the Bioterrorism (BT) Lab, Dr. Hom has been instrumental in the organization and coordination of all aspects of laboratory preparedness for responding to potential bioterrorism events, other disease outbreaks and other public health emergency threats and emergencies. He also is responsible for developing the Molecular Diagnostics Program. To date, rapid real time PCR assays utilizing either molecular beacon or TaqMan technologies have been developed and/or validated to detect various bacterial and viral pathogens that cause respiratory, tick-borne, and rash illnesses. Dr. Hom also has had extensive experience in the latest methodology employed for the confirmatory identification of "BT agents" such as *Bacillus anthracis* and ricin, West Nile Virus, and Eastern Equine Encephalitis Virus. Dr. Hom received his Ph.D. in Microbiology from the University of California, Davis and did Post-Doctoral work in Molecular Genetics at The Johns Hopkins University.

**NJ BIOTERRORISM RESPONSE:** Locally, the NJ State Public Health Laboratory is responsible for the organization and preparation of the frontline response to bioterrorism events. Included in this response is the coordinated interaction with other state and local facilities as part of the Laboratory Response Network. Dr. Hom's presentation will provide an overview of the various general functions and responsibilities of the public health laboratory while focusing on the laboratory's specific roles and capabilities in dealing with potential bioterrorism agents. Included in the discussion will be the logistics involved in the Laboratory Response Network, comments on specific bioterrorism bacterial and viral agents, available screening tests for these pathogens and the laboratory's recent experiences in dealing with the fall 2001 Anthrax outbreak.

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The cost of the dinner, payable at the door, is \$ 40 for members and \$ 50 for nonmembers.

Please be sure to get and wear your name tag when you register at the desk for dinner, as you will not be served dinner without it.

*All are invited to attend the lecture at 6 pm free of charge.*

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#### Directions to Crowne Plaza Hotel- Clark, NJ

**From Garden State Pkwy. (North/South):** Take exit 135 stay left - Northbound, take 2nd exit off circle - Southbound, take 4th exit off circle - Valley Rd. (toward Linden/Rahway). The Crowne Plaza is on your left.

**From NJ Turnpike (North/South) :** Take exit 11 to Garden State Pkwy. North, follow directions above for Parkway.

**From New York:** Take NJ Turnpike to exit 14. Take Rt. 78 West to the Garden State Pkwy. South to exit 135. Bear left off the exit, go around the circle to 4th exit on the right to Valley Rd. (toward Linden/Rahway). The Crowne Plaza is on your left.

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\*\*\*\*\***IMPORTANT NOTICE ON NEXT PAGE**\*\*\*\*\*

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Talk by Dr. Sherman Horn  
NJPHL LABS  
Clark, NJ 3/17/04

## New Jersey Public Health Laboratory Bioterrorism Response Activities

Sherman Horn, Ph.D.  
Director Bioterrorism Lab &  
Molecular Detection Services  
Dept. of Health & Senior Services  
Public Health & Environmental Labs  
March 17, 2004



## Summary

- Overview of Public Health Lab (PHL)
- Laboratory Response Network
- NJPHL Bioterrorism (BT) Lab Group
- BT Agents
- Methods to Detect BT Agents
- 2001 Anthrax Outbreak



## State PHL Roles

- Disease ID & outbreak investigation
- Reference services
- Environmental testing
- Clinical laboratory improvement
- Applied research
- Disease surveillance
- **Emergency Preparedness and Response**



## PHL Testing Services

- Bacteriology
  - Sexually transmitted disease bacteria
  - Enteric bacteria
  - Other bacteria (e.g. *Staphylococcus*;  
*Streptococcus*)
- Bioterrorism (BT)
  - Select agents and toxins



## PHEL Testing Services (cont.)

- Clinical Services
  - Drugs of abuse
  - Special Immunology
 (*Borrelia burgdorferi* & *Legionella pneumophila*)
- Mycobacteriology
  - Mycobacterium tuberculosis*



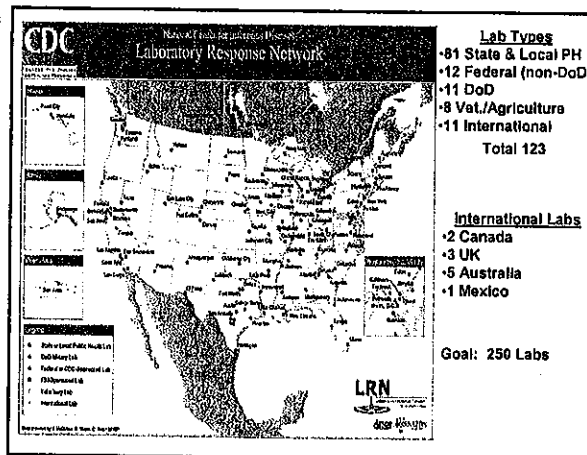
## PHEL Testing Services (cont.)

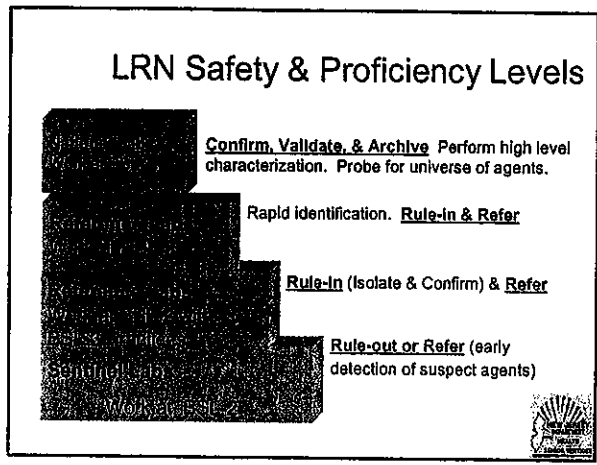
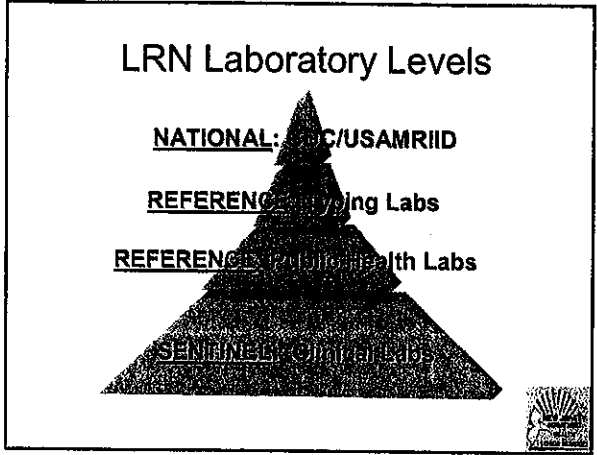
- Sanitary Bacteriology
  - Milk and water testing
- Virology
  - SARS CoV, HIV, hepatitis A/B/C, rabies, West Nile Virus, Influenza, etc.)



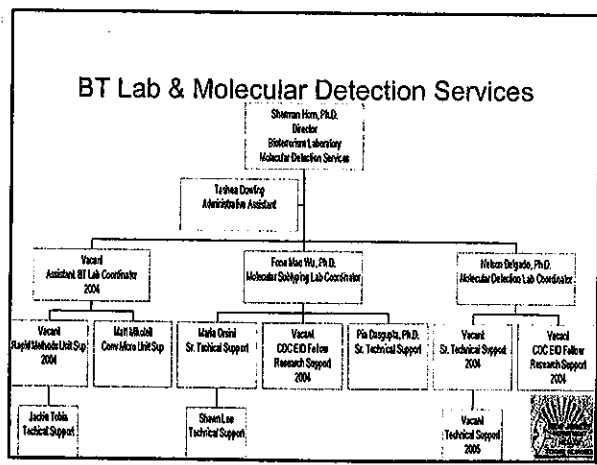
## Laboratory Response Network (LRN)

- Responds to BT events, disease outbreaks, and public health emergencies
- Includes public, private, & foreign labs
- Uses consensus test protocols
- Performs rapid & accurate testing & reporting
- Linked with local, state, and federal agencies
- Supported by CDC, APHL, and the FBI





- ### LRN Strategic Initiatives
- Increase Vet. Labs for animal & human health
  - Enhance Food Testing
    - Add 7 foodborne biothreat agents in '04
  - Increase Env. Labs for drinking H<sub>2</sub>O testing
  - Increase International Labs



## Facilities

- ❖ Present:  
1500 sq. ft. BSL-2  
(Use BSL-3 Practices)
- ❖ Late 2004 (Projected)  
3000 sq. ft BSL-3 (Modular)



## Category A Diseases/Agents

- Risk to national security
- Easily transmitted from person to person
- High mortality rates
- Major public health impact
- Cause public panic
- Require special action for PH preparedness



## Biological Diseases/Agents Category A

- Anthrax (*Bacillus anthracis*)
- Botulism (*Clostridium botulinum* toxin)
- Plague (*Yersinia pestis*)
- Smallpox (*Variola major*)
- Tularemia (*Francisella tularensis*)
- Viral hemorrhagic fevers  
Filoviruses – Ebola, Marburg



## Category B Diseases/Agents

- Moderately easy to disseminate
- Moderate morbidity rates
- Low mortality rates
- Require specific enhancements of PH Lab's diagnostic capacity



### Biological Diseases/Agents Category B

- Brucellosis (*Brucella* species)
- Epsilon toxin of *Clostridium perfringens*
- Food safety threats  
*Salmonella*, *E. coli* 0157, *Listeria*, ...
- Glanders (*Burkholderia mallei*)
- Melioidosis (*Burkholderia pseudomallei*)
- Q fever (*Coxiella burnetii*)



### Biological Diseases/Agents Category B (Cont.)

- Ricin toxin (castor beans)
- Staphylococcal enterotoxin B
- Typhus fever (*Rickettsia prowazekii*)
- Viral encephalitis  
alphaviruses – VEE, EEE, WEE
- Water safety threats  
*Vibrio cholerae*  
*Cryptosporidium parvum*



### Incubation Period of Likely BT Agents Time to Onset of Disease

Disease	Usual	Range
Anthrax	48 hr	Hours to days
Botulism	12-36 hr	Several days
Brucellosis	5-60 days	Several mos.
Plague	2-4 days	1-7 days
Smallpox	2 weeks	
Tularemia	3-5 days	1-14 days



### Category C Diseases/Agents

- Emerging or engineered pathogens
- High morbidity & mortality rates
- Major health impact
- Questions  
Availability?  
Ease of Production?  
Ease of Dissemination?



## Category C

- Emerging infectious disease threats  
Hantavirus
- Genetically engineered microbes  
*Bacillus cereus* w/*B. anthracis* tox genes
- Genetically engineered vectors containing human genes  
"Metabolic disruptors" →



## Pest Control Becomes "Life"Control

- Host: mouse (vac. against mousepox)
- Gene: mouse interleukin-4 reg. gene
- Vector: mousepox
- Experiment:  
mousepox/I-4 reg. gene → mouse = **death**
- Smallpox/hu I-4 reg. gene → human = ???



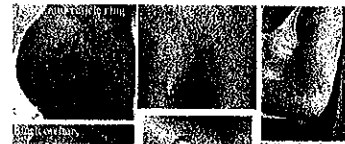
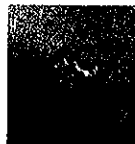
## Identification & Confirmation

- Microbiological  
Culture
- Microscopy  
Gram Stain
- Immunological  
Direct Fluorescent Antibody Test
- Molecular Diagnostics  
Real time PCR  
Timed Resolved Fluorescence



## Cutaneous Anthrax

Day 2



Notice the edema

and typical lesions

Exuberant granules

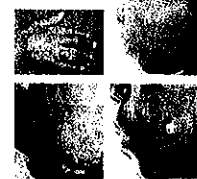
Day 10



Day 15



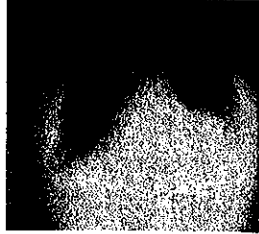
Scale of the



### Inhalational Anthrax



Mediastinal widening with inhalational anthrax



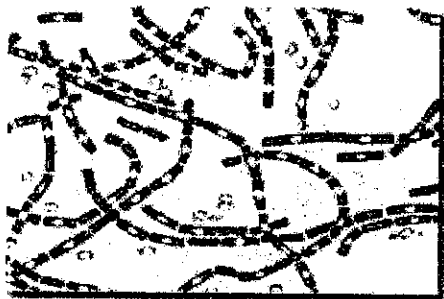
Mediastinal widening and pleural effusion on Chest X-Ray in inhalational anthrax



### *Bacillus anthracis* Culture



### *Bacillus anthracis* – Gram Stain



### *Bacillus anthracis*

- ❖ Confirmatory Testing (ES ~60 hr/CS ~6 hr)
  - DFA – capsule
  - DFA – cell wall
- ❖ Screening for cells & spores (~2-4 hr)
  - Real-time PCR





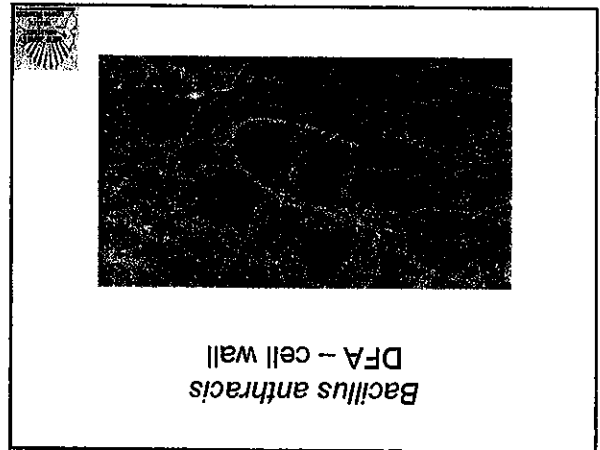
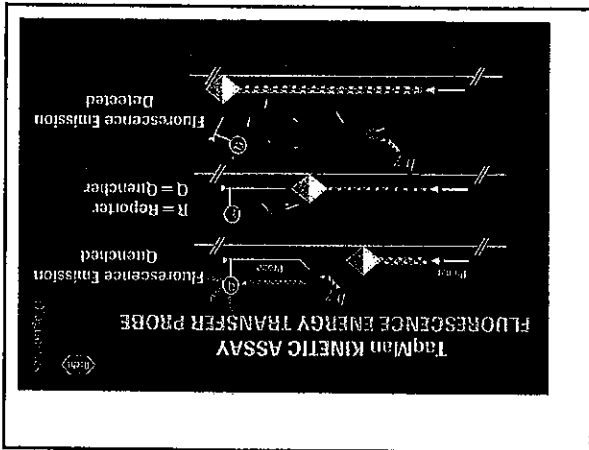
**2001 US Anthrax Outbreak**

- Sept. 22<sup>nd</sup> to Nov. 14<sup>th</sup> (onset date)
- 22 (18 confirmed) anthrax cases
- 11 inhalational (5 deaths)
- 11 cutaneous (7 confirmed)
- NY, NJ, FL, PA, VA, MD, CT
- 4 "Anthrax letters" delivered via USPS (USPS Hamilton Mail Processing Center)
- Princeton Letter Box → *B. anthracis* + \$64,000 Question: Exact location?

**PHL Sequence Detection Systems**

- Roche LightCycler (2 x 32)
- Cepheid Smart Cycler (2 x 16)
- ABI Prism 7000 (1 x 96)

Total Capacity: 768 rxns/8 hr

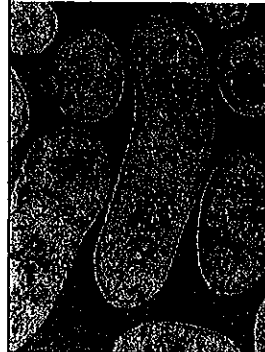


## NJ Samples Tested for "Anthrax"

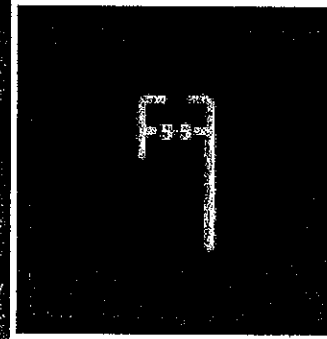
- Sample types
  - Environmental
  - Law enforcement investigations
  - U.S. Post Office Clean-up
  - Clinical
- 2001 ~3,500
- 2002 ~4,000
- 2003 ~7,500
- 2004 ~2,250 to date
- Total: 17,250



*Clostridium botulinum*



Botox



## *Clostridium botulinum*

- ❖ Botulinum Toxin (CS ~48 h/Food S ~72 h)
  - Mouse bioassay w/ & w/o neutralization with antitoxins
- ❖ *Clostridium botulinum*
  - Anaerobic growth
  - Gram stain
  - Egg yolk agar (lipase activity)

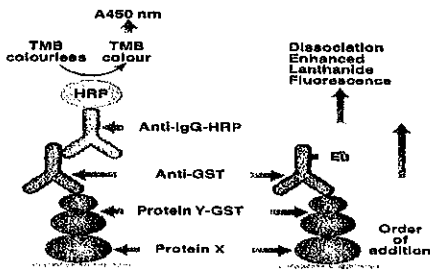


## Ricin (castor bean) Staphylococcal Enterotoxin B

- ❖ Test
  - Real time PCR (~2-4 hr)
  - Time resolved fluorescence (TRF; ~3-5 hr)

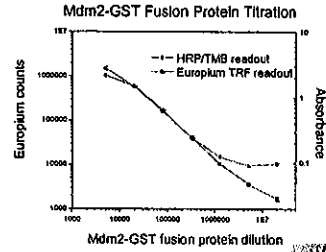


### Conversion from ELISA to DELFIA (TRF)

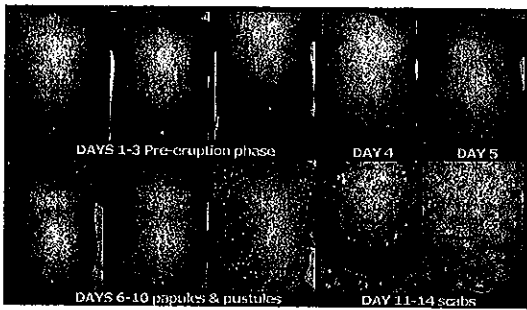


### DELFLIA® (TRF) vs. ELISA Detection

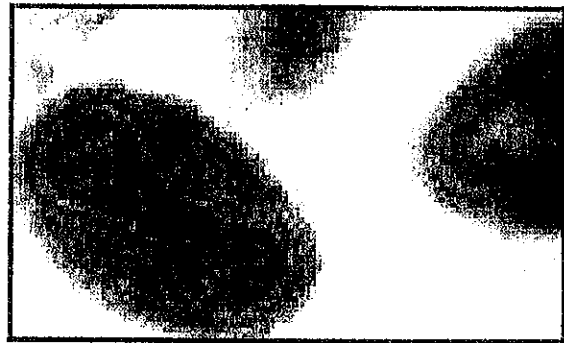
- Increased sensitivity
- Extended range
- Increased reproducibility



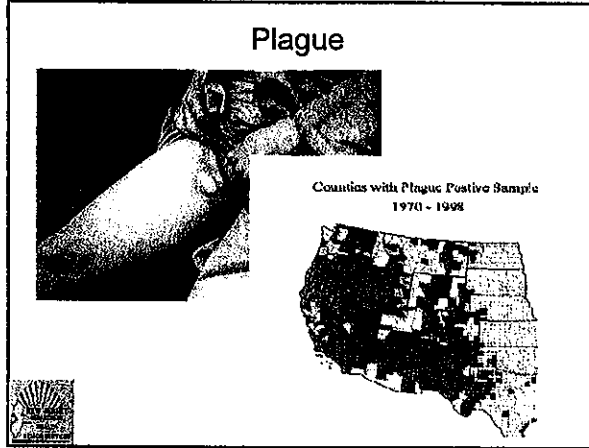
### Smallpox



### Smallpox Virus



Variola major Virus (Clinical Samples)		
Virus	Test Methods	Turn Around Time
Variola major	CDC (Real time PCR/EM)	Confirm ~12 h
Varicella zoster	PHEL (DFA Real time PCR)	Confirm ~7 h Screen ~2-4 hrs
Herpes simplex Type 2	PHEL (DFA Real time PCR)	Confirm ~7 d Screen ~2-4 hrs
Enterovirus	PHEL (DFA Real time PCR)	Confirm ~7 d Screen ~2-4 hrs



### *Yersinia pestis*

- ❖ Confirmatory Testing (ES ~3-4 d)
  - DFA – cellular F1 antigen
  - Phage lysis at 22-25 & 35-37 deg C
- ❖ Screening (~2-4 h)
  - Real-time PCR

### Tularemia

- Disease of wild animals
- Vectors: fleas, ticks, mosquitoes, & biting flies
- Infection sources: contaminated hay, water, infected animals & carcasses, & aerosolized particles

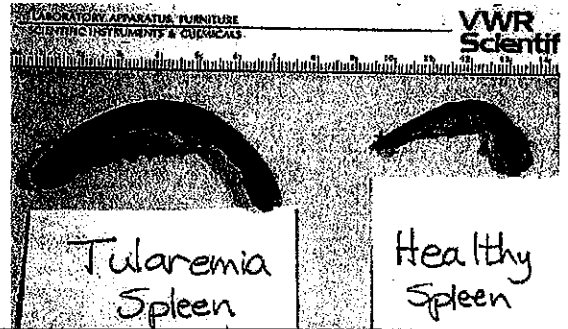
Figure 1. Reported cases of tularemia, United States, 1990-1999

Humans: ulceroglandular (45-80%)  
glandular infection (10-25%)  
oculoglandular (<5%)

## Tularemia



## Tularemia – Infected Spleen



## *Francisella tularensis*

- ❖ Confirmatory Testing (ES ~5-7 d)
  - DFA cellular surface antigens
  - Slide agglutination
  - Growth characteristics on agar media
  - Real-time PCR



## *Brucella & Burkholderia* spp. *Coxiella burnetii*

- ❖ Screen (*Brucella & Burkholderia*)
  - Biochemical Tests (~1-5 d)
  - Real-time PCR (~2-4 hr)
- ❖ Screen (*Burkholderia* only)
  - Antibiotic Susceptibility (Gn; ~3 d)
- Screen (*Coxiella* only)
  - Real-time PCR (~2-4 hr)





## Molecular Detection Services

- Validated Real time PCR assays
- Respiratory illnesses:
- Legionella pneumophila*
  - Legionella micdadei*
  - Legionella* spp.
  - Mycobacterium pneumoniae*
  - Chlamydia pneumoniae*



## Molecular Detection Services (Cont.)

- Validated Real time PCR assays
- Tickborne diseases:
- Borrelia burgdorferi* (Lyme)
  - Babesia microti* (Babesiosis)
  - Ehrlichia chaffeensis* (Ehrlichiosis)
  - Bartonella henselae* (Cat scratch fever) [?]
- Diarrheal Disease:
- Clostridium difficile* (Antibiotic-Associated Pseudomembranous Colitis)



## Bioterrorism Lab Websites

Search for:

Bioterrorism Resources

Search for:

Level A lab protocols