Today’s Presentation

- The Laboratory Response Network
- Sentinel Clinical Laboratory
- The Agents of Bioterrorism: A Review of the Sentinel Laboratory Protocols
“The LRN and its partners will maintain an integrated national and international network of laboratories that can respond quickly to acts of chemical or biological terrorism, emerging infectious diseases and other public health threats and emergencies.” — CDC.
The Laboratory Response Network

- Established by APHL, CDC and the FBI to improve US readiness for bioterrorism
- Interconnected system of laboratories
- Provide standardized testing in response to a bioterrorism event
The Laboratory Response Network

- Network of local, state and federal public health, hospital-based, food testing, veterinary and environmental testing laboratories.
  - Provide laboratory diagnostics
  - Have the capacity to respond to biological and chemical terrorism and other public health emergencies.
The Laboratory Response Network

- More than 150+ federal, state, and local labs in US and abroad
- National labs consist of CDC and Military
- Reference Labs are BSL-3 labs capable of confirming agents.
The Sentinel Clinical Laboratory

- Sentinel labs provide routine diagnostic services, rule-out and referral.
- Sentinel labs play a key role in the early detection of biological agents.
**Sentinel Clinical Laboratories**

- Definition of the Sentinel Clinical Laboratory developed by the Sentinel Laboratory Partnerships and Outreach Subcommittee of the Public Health Preparedness and Response Committee, Oct. 2012.
- The Lab is certified by CLIA or lab is a DoD certified under the DoD Clinical Laboratory Improvement Program or
- Lab is a veterinary diagnostic Lab, fully accredited by AAVLD
- Lab in-house testing includes Gram stains and at least one of the following:
  - Lower respiratory tract, wound or blood cultures.
Role of the Sentinel Laboratory

- Rule out critical biological agents
- Refer to higher level laboratory
LRN Structure for Testing and Specimen Flow

Level D Laboratory (CDC)

- Anthrax Lab
- Plague Lab
- Other Agent Specific Lab
- CDC The Core Lab Rapid Response & Advanced Technology Lab

SENTINEL LABORATORY

REFERENCE LABORATORY
The Agents of Bioterrorism
Sentinel Laboratory Protocols

- Protocols should be available to all staff--Copies are available at the American Society for Microbiology web site [www.asm.org](http://www.asm.org)
- ASM Sentinel Laboratory Guidelines, updated July 2013.
- Integrate protocols into laboratory SOP and review along with other laboratory documents.
## Bioterrorism Agents: Laboratory Risk

<table>
<thead>
<tr>
<th>Agent</th>
<th>BSL spec/culture</th>
<th>Laboratory Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. anthracis</td>
<td>2/2</td>
<td>low</td>
</tr>
<tr>
<td>Y. pestis</td>
<td>2/2</td>
<td>medium</td>
</tr>
<tr>
<td>Brucella spp.</td>
<td>2/3</td>
<td>high</td>
</tr>
<tr>
<td>F. tularensis</td>
<td>2/3</td>
<td>high</td>
</tr>
<tr>
<td>Botulinum toxin</td>
<td>2/2</td>
<td>medium</td>
</tr>
<tr>
<td>Smallpox</td>
<td>4/4</td>
<td>high</td>
</tr>
<tr>
<td>Viral Hemorrhagic fever</td>
<td>4/4</td>
<td>high</td>
</tr>
</tbody>
</table>
Dallas Capabilities

- B. anthracis (PCR/Culture)
- Y. pestis (PCR/Culture)
- F. tularensis (PCR/Culture)
- Brucella spp (PCR/Culture)
- Burkholderia spp (PCR/Culture)
- Orthopox/Non-orthopox virus (PCR)
Dallas Capabilities cont.

- C. burnetti (PCR only)
- Ricin (TRF and PCR)
- Varicella (VZV, DFA only)
- Influenza A/H5, H1N1, H7N9, H3N2(v) & Seasonal
- West Nile Virus
- Food/Clinical-Salmonella, Listeria, E coli
- Food-BT agents and Shigella
Dallas Capabilities cont.

- Norovirus (outbreaks only)
- Respiratory Panel
  - RSV
  - Parainfluenza virus 1,2,3
  - Adenovirus
Bacillus anthracis

ANTHRAX
In a Sentinel Laboratory you might encounter this organism in:

- Direct specimen smear
- Blood culture or CSF (inhalational, septicemic)
- Vesicle fluid, swab or biopsy of eschar (cutaneous)
- Stool (gastrointestinal)
Perform all testing in a BSC

- Gram stain
- Culture characteristics on agar
- Hemolysis
- (Motility)
- (Catalase)
Gram stain morphology in smears from clinical specimens

- Gram-positive rods; short chains of two to four cells
- Spores are not normally observed in smears from clinical specimens
Gram Stain of Spinal Fluid

345(22):1607-1610
Gram Stain Morphology from Culture

- Large Gram-positive rod
- Usually non-encapsulated, often in long chains
- Cells are more easily decolorized with age
Gram Stain Morphology from Culture

- Oval spores, central to sub-terminal, with no swelling of cell
- Spores increase with age of culture
Gram Stain of *B. anthracis* from Culture
Culture characteristics

- Organism will grow on most routine culture media---SBA, CHOC, but not MAC
- Will grow in routine blood culture systems
- Growth on plate media may be evident as early as 8 hours
Colony on SBA at 35°C, 18-24hr

- Colonies are round with irregular edges, flat or slightly convex with ground glass appearance
- Undulate edge may show curling resembling a “Medusa head”
- “Tenacious” or “sticky”
B. anthracis colonies on SBA
B. anthracis colonies on SBA
Hemolysis

- *B. anthracis* is non-hemolytic
B. anthracis colonies on SBA

B. cereus

B. anthracis

B. cereus
Sentinel Laboratory Procedures for *B. anthracis*

**Motility**

- Motility – should be negative.
Motility Test Medium

B. cereus  B. anthracis
Sentinel Laboratory Procedures for *B. anthracis*

*Bacillus* spp. are catalase positive—**CAUTION** test produces aerosol
“B. cereus” Group Characteristics

- Ground glass colony/ non-swelling, sub-terminal spores—*B. anthracis*, *B. cereus*, *B. cereus* var. *mycoides*, and *B. thuringiensis*
- Non-motile—*B. anthracis* and *B. cereus* var. *mycoides* (and *B. megaterium*)
- Non-hemolytic—presumptive *B. anthracis*
Bacillus anthracis Technical Clues

IF YOU SEE:

- Rapidly growing, flat, “ground-glass” colonies on SBA
- Large Gram-positive, aerobic rods
- Non-hemolytic
- Non-motile
- (Catalase positive)
REFER ISOLATE TO DALLAS COUNTY LRN REFERENCE LABORATORY
Francisella tularensis

TULAREMIA
This is a dangerous, highly virulent organism and it should not be manipulated on an open bench. Laboratory-acquired infections can occur.
In a Sentinel Laboratory you might encounter this organism in:

- Direct specimen smear
- Blood culture
- Wound (ulcer) biopsy or swab (not preferred)
- Lymph node aspirate (ulceroglandular)
- Sputum or bronchial washings (inhalational)
Sentinel Laboratory
Procedures for *F. tularensis*

- Gram stain
- Culture characteristics
- Oxidase test
- Urease activity
- (Catalase test)
- beta-lactamase test
- XV or satellite test
Gram Stain morphology

- Very small gram-negative coccobacillus
- May stain weakly
- Fastidious organism, requires cysteine
- Difficult to see individual cells
Gram Stain of

*Francisella tularensis*
Gram Stain of
*Francisella tularensis*
Culture characteristics

- Slow growing, nutritionally fastidious
- Grows on SBA initially, poorly or not at all on subculture
- Cysteine-enriched media such as CHOC, TM, BCYE, thioglycollate broth support subculture.
Culture characteristics @ 35°C

- On SBA, CHOC, BCYE at 8 – 24 hrs: gray-white, translucent colonies usually too small to be seen individually

NOTE: F. tularensis grows poorly at 25°C – this is good in differentiating from Y. pestis, which grows better at 25°C.
Sentinel Laboratory Procedures for *F. tularensis*

Growth on SBA at 24 - 48 hours
Sentinel Laboratory Procedures
for *F. tularensis*

**Culture characteristics at 35° C**

- 48 hours on SBA: 1-2 mm, gray-white to bluish grey, opaque, no hemolysis
- 48 hrs on CHOC, BCYE: 1-2 mm, gray-white, opaque, entire, smooth, shiny
- Mature growth at 72 hrs
Growth Characteristics of *F. tularensis*

Growth on chocolate agar at 48-72 hours
Growth Characteristics of *F. tularensis*

Growth on chocolate agar at 72 hours
Sentinel Laboratory Procedures for *F. tularensis*

Biochemical Tests

- Oxidase - negative
- Urease - negative
- Catalase - weakly positive or negative

**CAUTION** -- test produces aerosol

- beta-lactamase - positive
- XV factor not required
Most likely:
- *Acinetobacter* (oxidase negative)
- *Actinobacillus* spp.
- *H. aphrophilus / H. influenzae*
- *Bordetella*, Grp. IV (inert, urea pos)
- *Pasturella* (nonsticky, MAC +)

Least likely:
- *Dysgonomononas* spp. (DF-3)
- *Brucella* spp.
Francisella tularensis
Technical Clues

IF YOU SEE:

- 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 hours
  - Oxidase (-), catalase (wk+ or -), urease (-), XV or satellite (-), beta-lactamase (+)
REFER ISOLATE TO DALLAS COUNTY LRN REFERENCE LABORATORY
Yersinia pestis

PLAGUE
In a Sentinel Laboratory, you might encounter this organism in:

- A direct specimen smear
- Blood culture (septicemic)
- Lymph node aspirate (bubonic)
- Respiratory culture (inhalational)
Sentinel Laboratory Procedures for *Y. pestis*

- Gram stain morphology
- Culture characteristics
- Oxidase test
- Urease test
- Indole test
- (Catalase test)
- (Culture characteristics in broth)
- (Wayson or Giemsa / Wright’s stain)
Gram Stain morphology

- “Large” Gram-negative rod
- Resembles other *Enterobacteriaceae*
Gram Stain of *Y. pestis*
Giemsya or Wayson stain morphology

- Dark blue rod-shaped organisms
- From direct specimen material may show “safety-pin” morphology
- “Safety-pin” feature is neither specific nor sensitive
Wright-Giemsa Stain of Y. pestis
Culture characteristics

- Will grow on routine culture media--SBA, CHOC, and MAC
- Resembles other *Enterobacteriaceae*, *EXCEPT* grows faster at 28°C or RT than at 35°C
- Grows in routine blood culture systems
Sentinel Laboratory Procedures for Y. *pestis*

Biochemical results analysis:

- **Oxidase-negative** results distinguishes *Y. pestis* from bipolar-staining *Pasteurella* species
- *Yersinia pestis* is the only non-motile species of the Yersiniae
- **Urease-negative** results distinguishes *Y. pestis* from *Y. pseudotuberculosis*
Y. pestis on SBA
Y. pestis in Broth Culture

Y. pestis
Y. pseudotuberculosis
Sentinel Laboratory Procedures for *Y. pestis*

Biochemical Tests

- Oxidase - negative
- Urease - negative
- Indole - negative
- Catalase – positive
  --CAUTION-- catalase test produces aerosol
Y. pestis Technical Clues

IF YOU SEE:

- Gram-negative rods from blood, lymph node aspirate, or respiratory specimens
  - Pinpoint colonies at 24 h on SBA
- Colonies resemble enterics, but grow better at 28°C than at 35°C
- Non-lactose fermenter on MAC
- Catalase (+), oxidase (-), urease (-), and indole (-)
REFER ISOLATE TO DALLAS COUNTY LRN REFERENCE LABORATORY
Brucella spp.

BRUCELLOSIS
Brucellosis has been the most commonly reported laboratory-associated bacterial infection, aerosols are highly infectious.

Cases have occurred in clinical laboratory settings by “sniffing” cultures, direct skin contact with cultures, and aerosol generating procedures.
In a Sentinel Laboratory, you might encounter this organism in:

- Direct specimen smear
- Blood or bone marrow
- Tissue (spleen, liver)
Sentinel Laboratory Procedures for *Brucella* spp.

- Gram stain morphology
- Culture characteristics
- Oxidase test
- Urease activity
- (Catalase test)
- Satellite or X and V factors
Gram stain morphology

- Very tiny, faintly staining, Gram-negative coccobacilli
- Larger than *F. tularensis*--discrete organisms evident
Brucella spp. Gram Stain
Culture characteristics

- Will grow on SBA, CHOC, (MAC delayed)
- Organism will grow in routine blood culture systems, but may require extended incubation
Colony morphology on SBA at 35°C:

- Small (0.5-1.0mm), convex, glistening
- Non-hemolytic and non-pigmented
- Fastidious
- Visible growth may take 48 - 72 hrs
- Tiny gram negative CB’s
Brucella spp. Growth on SBA

Growth on SBA at 24 – 48 hours
Brucella spp. Growth on SBA

Growth on SBA at 72 hours
Biochemical Tests

- Oxidase-positive
- Urea hydrolysis-positive (*B. suis, B. canis* ~15 min--*B. abortus* and *melitensis* ~24hr)
- Catalase positive

CAUTION--test produces aerosol
Brucella spp. Urease Activity
Sentinel Laboratory Procedures for *Brucella* spp.

Serum - not for culture

The diagnosis of brucellosis is often made by serologic testing. An acute and convalescent phase specimen should be collected.
Organisms Resembling *Brucella* spp.

- Achromobacter grp B
- Acidovorax spp.
- Agrobacterium spp.
- CDC Group EO-2/EO-3
- Flavobacterium spp.
- Methylobacterium spp.

- Ochrobactrum anthropi
- Riemerella
- Roseomonas spp
- CDC Group O-2
- CDC Group II-i
- Oligella urealytica
Brucella spp. Technical Clues

IF YOU SEE:

- Tiny, faintly staining, Gram-negative coccobacilli from blood, bone marrow, or lymphoid tissue
- Slow growth on SBA, CHOC needing 2-3 days for colonies to appear
- Oxidase (+), urease (+), catalase (+)
- No satellite phenomenon (or XV factor negative)
REFER ISOLATE TO DALLAS COUNTY LRN REFERENCE LABORATORY
Burkholderia spp.

MELLIOIDOSIS / GLANDERS

Laboratory Exposure to Burkholderia pseudomallei---Los Angeles, California, 2003  MMWR Vol 53, No 42;988  10/29/2004
In a Sentinel Laboratory, you might encounter this organism in:

- Direct specimen smear
- Blood or bone marrow
- Sputum
- Abscess fluid or wound swab
- Throat or nasal swab
- Urine
Gram Stain of *Burkholderia mallei*
Gram Stain of *Burkholderia pseudomallei*
Culture Characteristics

- *Burkholderia pseudomallei* will grow on SBA, CHOC, MAC producing mature colonies at 48 to 72 hrs.

- *Burkholderia mallei* will also grow on SBA and CHOC, possibly on MAC, producing mature (small) colonies at 72 hrs.
B. mallei Growth on SBA @ 35°C
B. mallei Growth on SBA @ 35°C
B. pseudomallei Growth on SBA @ 35°C
B. pseudomallei Growth on SBA @ 35°C
B. pseudomallei Growth on SBA @ 35°C
Culture on CHOC at 35°C

- *Burkholderia mallei* – slow growth, mature colonies by 48 to 72 hrs
- *Burkholderia pseudomallei* – mature growth at 24 hrs
Burkholderia spp. Growth on CHOC @ 35°C

B. mallei

B. pseudomallei

24 hrs

48 hrs

72 hrs
Sentinel Laboratory Procedures for *Burkholderia* spp.

Culture on MAC at 35°C

- *Burkholderia mallei* – no growth, or very small @ 72 hrs
- *Burkholderia pseudomallei* – slow growth, producing colorless colonies with slight pink centers at 72 hrs
Burkholderia spp. Growth on MAC @ 35°C

<table>
<thead>
<tr>
<th>Time</th>
<th>B. mallei</th>
<th>B. pseudomallei</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>48 h</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>72 h</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
B. pseudomallei Growth on MAC
Biochemical Tests--oxidase

- *Burkholderia mallei* – oxidase variable
- *Burkholderia pseudomallei* – oxidase positive
Biochemical Tests—both organisms are:

- Indole negative
- Colistin resistant
- Catalase positive

**CAUTION** --test produces aerosol
Motility

- *Burkholderia mallei* – non-motile
- *Burkholderia pseudomallei* – motile
Burkholderia spp. in Motility Medium
Optional screening tests: Growth in triple sugar iron (TSI) agar:

- *Burkholderia mallei* – no change, slant or butt
- *Burkholderia pseudomallei* – no change, slant or butt, or acid over no change
*Burkholderia spp.* on TSI Agar
Sentinel Laboratory Procedures for *Burkholderia spp.*

Optional screening tests: arginine dihydrolase

- Both organisms are positive
Optional screening tests: reduction of nitrate

- *B. mallei* reduces nitrate w/o gas
- *B. pseudomallei* reduces nitrates to gas
Organism Resembling *Burkholderia spp.*

- *Burkholderia cepacia*
- *Burkholderia gladiolii*
- *Pseudomonas mendocina*
- *Pseudomonas stutzeri*
- *Ralstonia picketii*
- *Stenotrophomonas maltophilia*
B. pseudomallei Technical Clues

IF YOU SEE:

- Gram-negative aerobic rods
- Characteristic slow to moderate growth on SBA and MAC
- Oxidase positive
- Motile
- Catalase positive
IF YOU SEE:

- Gram-negative coccobacilli
- Characteristic very slow growth on SBA and little if any growth MAC
- Oxidase variable
- Non-motile
- Catalase positive
REFER ISOLATE TO DALLAS COUNTY LRN REFERENCE LABORATORY
BOTULISM TESTING

Stool or Serum
Botulinum toxin

- The suspicion of botulism is a public health emergency: notify both local public health officials and the state public health laboratory for approval to submit samples for testing. Submit specimens without delay.
Botulinum toxin

- Do not attempt to isolate the organism from clinical specimens
Other Agents—Toxins

Botulinum toxin specimens

- Feces, enema fluid
- Gastric aspirate or vomitus
- Serum
- Tissue or GI contents collected post morte
Botulinum toxin specimens

- Hold all specimens at 4°C and transport to LRN Reference Laboratory as soon as possible
Other Agents--**Toxins**

Botulinum toxin specimens—**not** to be processed by Sentinel Laboratories

- Surface swabs
- Food
- Soil or water
Specimens Submitted For Botulism Testing

- Patient history
  - We need a clinical history
- Patient should present with impaired nerve function
- Adults typically have symmetrical descending paralysis
- Infants can have a wide range of symptoms, but many are hypotonic and constipated
- Wound botulism may not be obvious
Types of Specimens

- **Stool**
  - 10-50 grams recommended for an adult
  - At least 5 grams for an infant.

- **Serum**
  - 10 ml minimum for an adult is recommended
  - Testing for infants is not recommended
Types of Specimens

- **Wound**
  - Tissue from a biopsy or swab from deep in the wound

- **Food**
  - Only tested if associated with a confirmed botulism case
Shipping Conditions

- Stools and serum
  - Should be shipped cold (on cold packs) by overnight courier
- Wound
  - Ship tissue in anaerobic atmosphere
  - Swab in anaerobic transport for swabs
- Food
  - Should be shipped in original container
  - Should be shipped according to storage
We Need This Information

- Is this STAT?
- What is the neurological history?
- What type of sample are you sending?
- How will the samples be delivered?
- When will it arrive?
- Physician contact name and phone number
Diagnosing Botulism

- Symptoms include:
  - Flaccid paralysis, descending bilateral paralysis
  - Droopy eyelids, double vision
  - Slurred speech, difficulty swallowing and respiratory collapse

- Wound botulism can occur due to traumatic injuries or illicit drug use
Infant Botulism

- The most common form
- Colonizes the gut and toxin is formed in vivo. Symptoms:
  - 100% limb weakness (floppy baby syndrome)
  - 60% sluggish pupils
  - 78% bulbar weakness
  - 64% require assisted ventilation
Toxin

- Finding toxin in serum is difficult
- It is frequently negative
- Stool is the specimen of choice
Toxin Types

- Types A, B, E, and F cause illness in humans
- Type A is most common in West Texas
  - order of toxicity, F most toxic > C > A > D > B > least toxic
- Type B is more common in East Texas, but either type can be found anywhere
- Botulism is probably substantially under diagnosed
- The neurotoxin of *C. botulinum* is considered to be a select agent
Reporting

- Five days minimum for a negative report (Austin)
  - We always call with any positive results
- If physician strongly suspects botulism in an adult, antitoxin can be ordered (Austin)
  - **Physician must contact DSHS Epidemiologist and or Dallas County Chief Epidemiologist for consult.**
- Primary contact for Dallas County Health Lab:
  - Blake Myers, 214-819-2840 (office); 972-342-5605 (24/7 cell)
  - Dr Wendy Chung, 214-642-8080 (24/7 cell)
- Primary contact at DSHS, Biothreat Team 24/7,
  - 512-689-5537 (24/7 cell)
Role of the Sentinel Laboratory

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

OR

- REFER to your LRN Reference Laboratory
QUESTIONS?