Wisconsin State Laboratory of Hygiene

UNIVERSITY OF WISCONSIN–MADISON

Case Study

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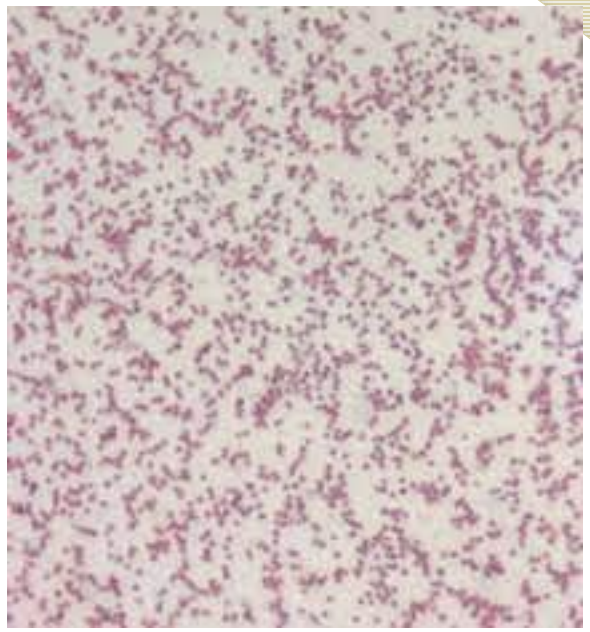
Case- Presentation

- 24 year old male
- Wound on his arm from a recent cat bite that won't heal
- Fever, chills, body aches
- Specimen taken for culture
- Antibiotics prescribed

Case- Work-up

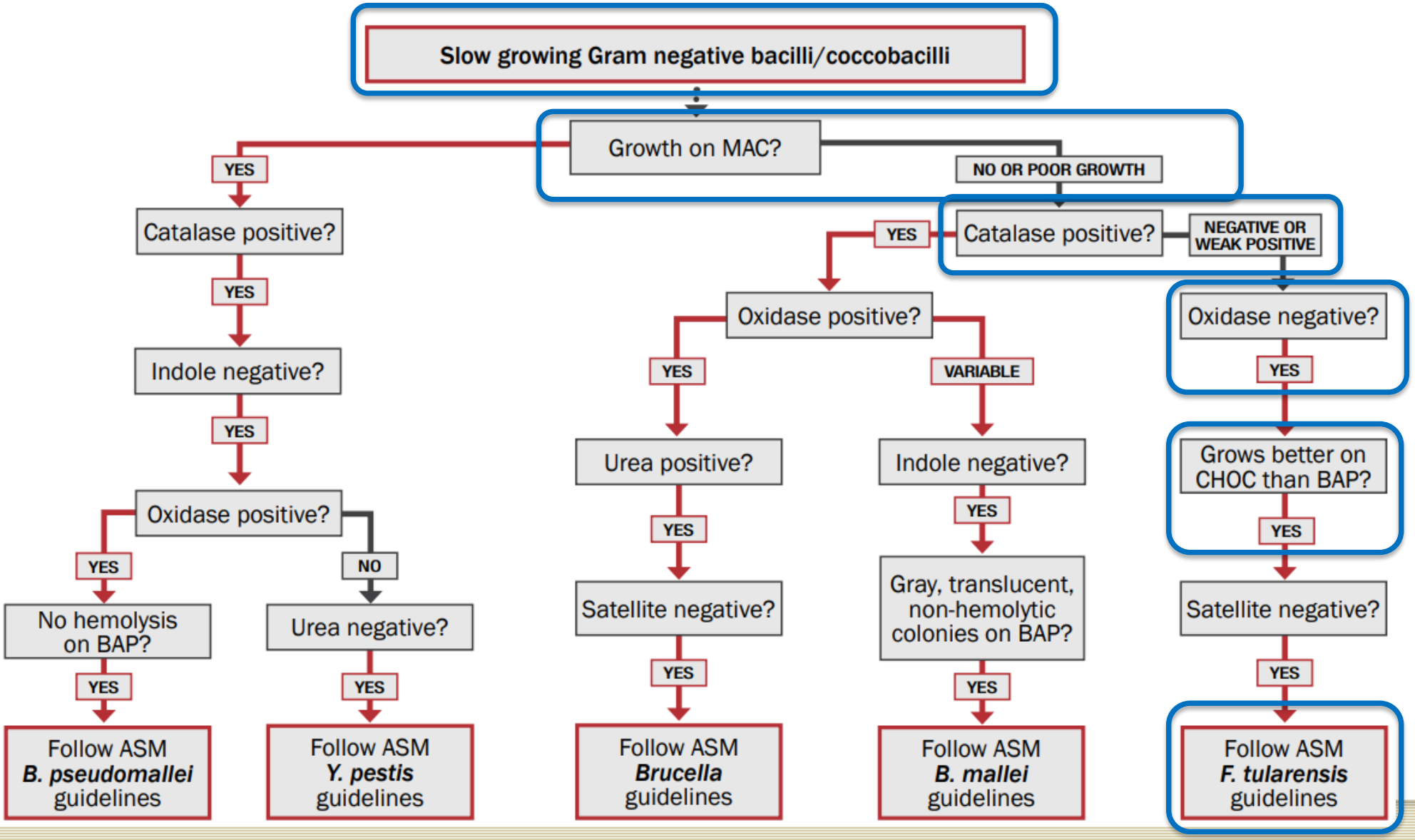
- Gram stain shows poorly staining gram negative bacilli
- Cultures are slow to grow.
  - Pinpoint colonies seen on chocolate agar after 48 hours.
  - Very light growth on BAP in the first quadrant only
  - No growth on MAC.



https://www.aphl.org/aboutAPHL/publications/Documents/2018_BiothreatAgents_SentinelLab_BenchCards_WEB.pdf

BIOTHREAT AGENT IDENTIFICATION

Gram Negative Bacilli/Coccobacilli Rule-Out Algorithm



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TULAREMIA — *Francisella tularensis*

Rule-Out Algorithm

SAFETY

As soon as *Francisella* is suspected, **perform all further work in a Class II BSC** using BSL-3 practices. If *F. tularensis* cannot be ruled out with tests below, **do not attempt further ID** using commercial automated or kit identification systems.

Gram stain morphology

- ☐ Pleomorphic?
- ☐ 0.2–0.5 µm by 0.7–1.0 µm faintly staining, Gram negative coccobacillus?
- ☐ Mostly single cells?

Colony morphology

- ☐ Aerobic and fastidious?
- ☐ No growth on MAC/EMB
- ☐ Scant to no growth on BAP after 48h? **Note:** may grow on primary BAP culture, but not on subculture.
- ☐ Slow growth on CHOC, TM or BCYE?
- ☐ 1-2 mm gray to grayish-white colonies on CHOC after 48h
- ☐ Colonies opaque, grey-white, butyrous with smooth and shiny surface?

YES TO ALL

24h: Growth on CHOC but not BAP?
48h: Growth better on CHOC than BAP?

NO TO ANY / NO

YES

Are colonies satellite negative?

NO

YES

Oxidase negative and either catalase weakly positive or negative?

NO

YES

β-lactamase positive?
No growth on MAC?

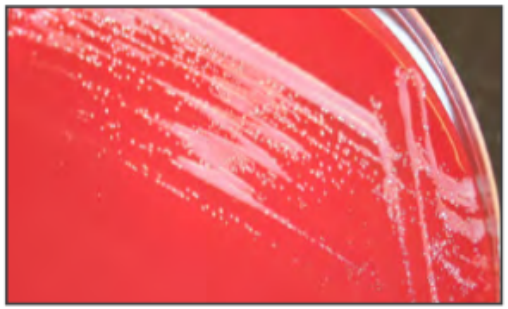
NO

YES, STOP

Francisella tularensis **ruled out.** Continue with routine identification

Consider *Haemophilus*

***Francisella tularensis* not ruled-out.** Do not attempt further identification and contact your LRN Reference Level Laboratory to refer the isolate. **Suggested Reporting Language:** Possible *F. tularensis* submitted to LRN Reference Level Laboratory for confirmatory testing.



48h growth on BAP

48h growth on CHOC

LRN™
LABORATORY RESPONSE NETWORK

Accurate and timely diagnosis and detection of chemical and biological threat agents anywhere in the United States

LRN-B: Biological

- 150+ Member Labs
- 84% of Americans live within 100 miles of a lab
- 45 distinct tests for biological threats
- ~3,000 specimens processed in 2017

Network Labs

Federal—These are labs at CDC, the U.S. Department of Agriculture (USDA), the Food and Drug Administration (FDA), and other facilities run by federal agencies.

Military—Department of Defense laboratories are operated both within the United States and abroad.

Food testing—The LRN includes FDA and USDA labs, and others that are responsible for ensuring the safety of the food supply.

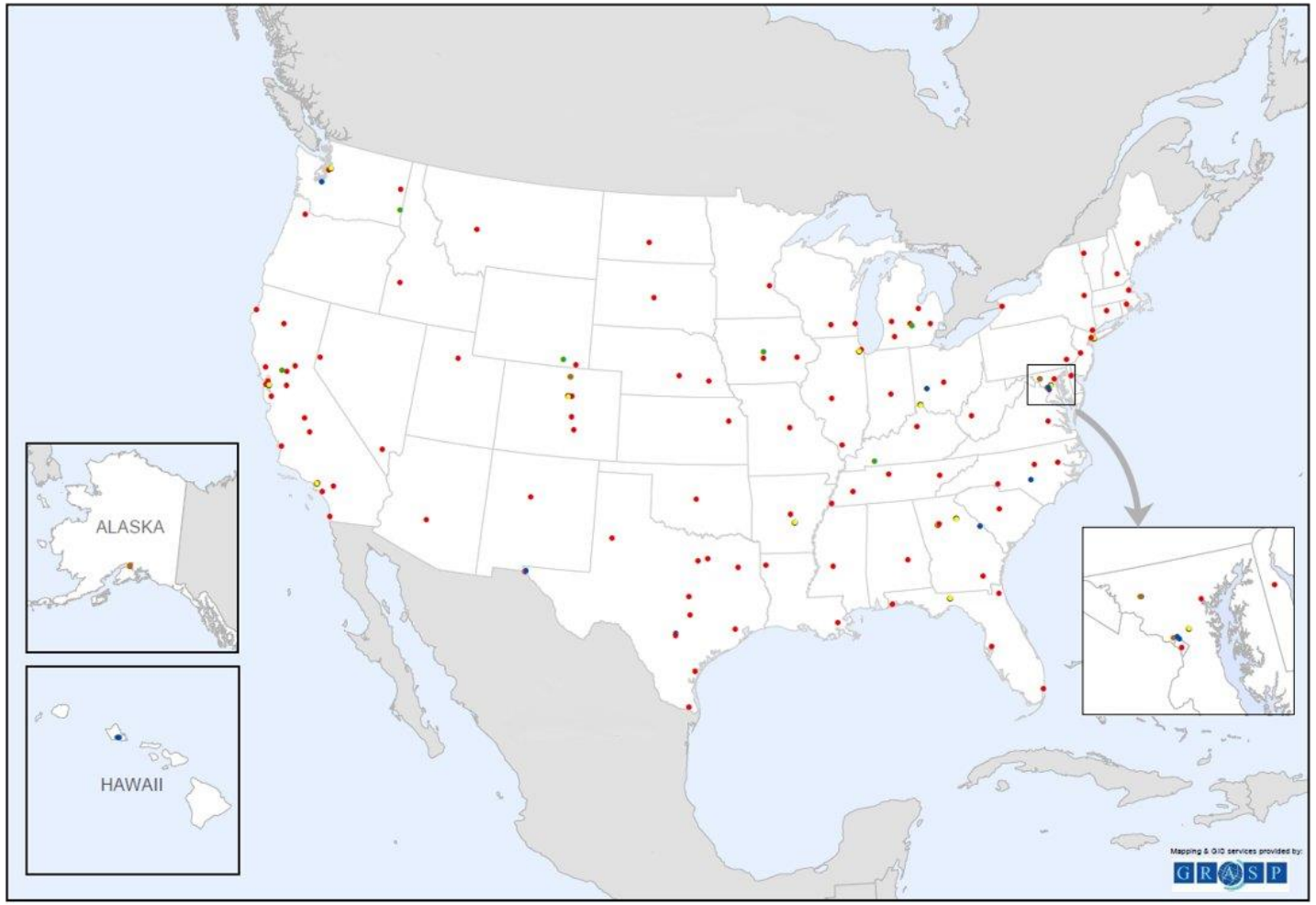
Environmental—These are labs that are capable of testing water and other environmental samples.

Veterinary—Some LRN labs, such as those run by USDA, are responsible for animal testing. Some diseases can be shared by humans and animals, and animals often provide the first sign of disease outbreak.

International—The LRN has several international partners who provide various levels of testing capabilities.

State and local public health—These labs are run by state and local departments of health. In addition to being able to test for biothreat agents, a few LRN public health labs are able to measure human exposure to toxic chemicals through tests on clinical specimens.

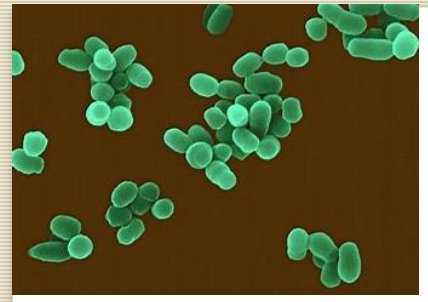
LRN-B Labs Across the US



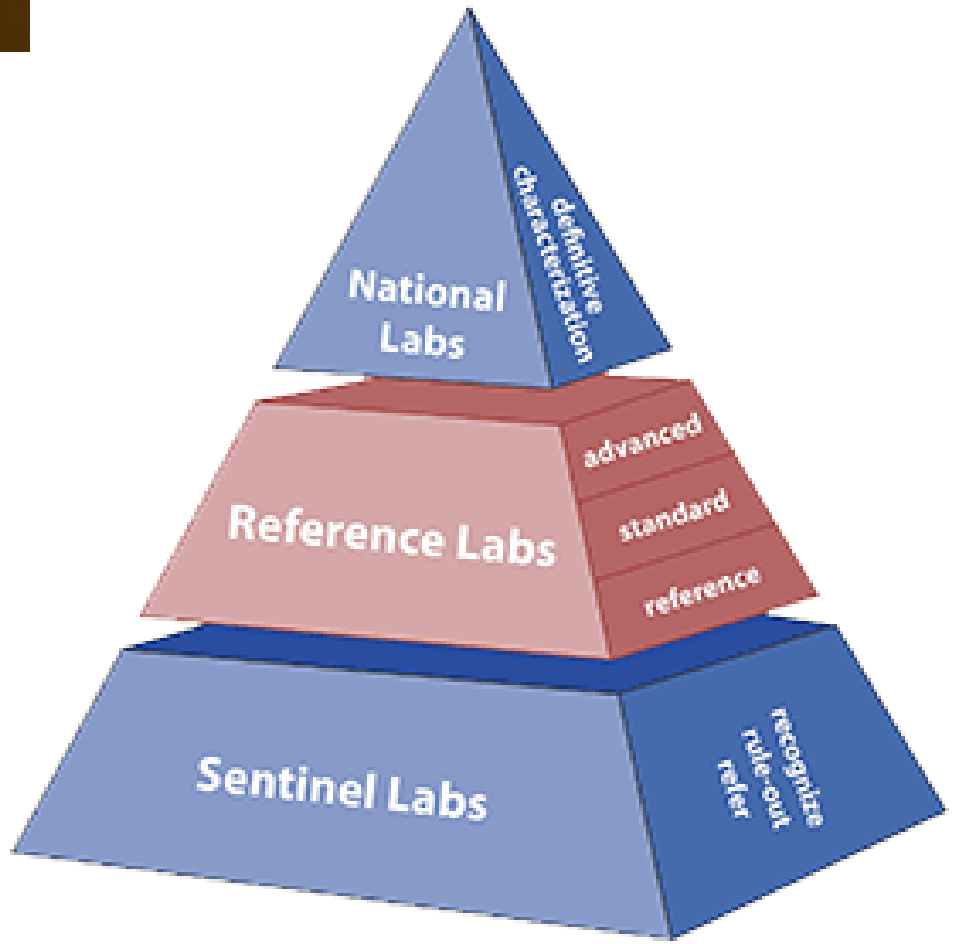
*At least one in every state, 120+ total labs

LRN-B Mission

- To provide a rapid laboratory response to biological threats to inform critical decisions about public health and safety
- Produce Data for Smart Public Health Decisions
- Maintain and Improve National Laboratory Capacity
- Strengthen National Security
- Test for Old and New Diseases
- Ensure High Standards, Enable High Confidence in Results



LRN-B Network Structure



"B. melitensis, genetically related to X outbreak"

"Brucella melitensis"

"Unable to rule out Brucella"

Case- Escalation

- Clinical Lab escalates the case
  - Consult with the clinician to see if this could be a select agent
    - Informs them of cat bite history
  - Calls the State Lab to notify of incoming shipment
    - Isolate sub-cultured and shipped to WSLH as category B
  - Calls their local or state health department to notify them of a suspect case

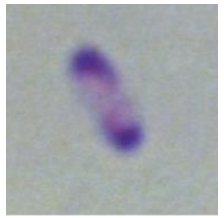
What we Test

- Clinical isolates when sentinel labs are unable to rule out select agents
- Primary specimen in highly suspect patients
- Environmental materials like suspicious white powders

Select Agents

Bacterial

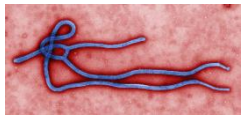
- *Bacillus anthracis**
- Botulinum neurotoxin producing species of *Clostridium**
- *Bacillus cereus* Biovar *anthracis**
- *Brucella abortus*
- *Brucella melitensis*
- *Brucella suis*
- *Burkholderia mallei**
- *Burkholderia pseudomallei**
- *Coxiella burnetii*
- *Francisella tularensis**
- *Rickettsia prowazekii*
- *Yersinia pestis**



Toxins

- Ricin
- Saxitoxin
- Staphylococcal enterotoxins A,B,C,D,E subtypes
- T-2 toxin
- Tetrodotoxin

Viral



- Hendra virus
- Nipah virus
- Rift Valley fever virus
- Venezuelan equine encephalitis virus
- Crimean-Congo haemorrhagic fever virus
- Eastern Equine Encephalitis virus
- Ebola virus*
- Lassa fever virus
- Lujo virus
- Marburg virus*
- Monkeypox virus
- 1918 Spanish flu
- SARS-associated coronavirus (SARS-CoV)
- South American Haemorrhagic Fever viruses:
  - Chapare virus
  - Guanarito virus
  - Junin virus
  - Machupo virus
  - Sabia virus
- Tick-borne encephalitis complex (flavi) viruses:
  - Far Eastern subtype
  - Siberian subtype
- Kyasanur Forest disease virus
- Omsk hemorrhagic fever virus
- Variola major virus (Smallpox virus)*
- Variola minor virus (Alastrim)*

* Tier 1 agent

https://www.selectagents.gov/SelectAgentsandToxinsList.html

Tier 1 Human Agents

- *Yersinia pestis*
- *Bacillus anthracis*
- *Bacillus cereus* Biovar *anthracis*
- *Burkholderia mallei*
- *B. pseudomallei*
- *Francisella tularensis*
- Botulinum neurotoxins
- Botulinum neurotoxin-producing Clostridium

- Ebola virus
- Marburg virus
- Variola major (Smallpox)
- Variola minor (Alastrim)

Case- Confirmatory testing

- State Lab tests for select agents
  - In a high containment, select agent registered, BSL3 lab
  - Gram stain, cultures, biochemicals, and PCR
  - All shipping and testing is fee exempt

Case- Reporting

- Positive results reported
  - By phone and final report to the submitting laboratory
    - Clinical labs notifies the doctor
  - To WEDSS (local and state) public health departments
  - To CDC LRN program

Case- Response

- Clinician contacts patient to get them on appropriate therapy
- Clinical Lab works with WSLH and DHS to determine if there have been any laboratory exposures.
- Local and/or state health departments contact patient for the case investigation

Case- Investigation

The case of Francisella via bite from paralyzed parturneuriant cat

Case- Conclusion

- WSLH sends isolate to CDC in case additional testing is needed
- Lots of Forms (4 and 2)
- Clinical Lab destroys all materials within 7 days
- CDC consulted about the need to destroy the cats
- FBI not notified as the source was considered a “routine” environmental exposure and not a biothreat

Resource

Biothreat Agent Bench Cards for the Sentinel Laboratory
https://www.aphl.org/aboutAPHL/publications/Documents/2018_BiothreatAgents_SentinelLab_BenchCards_WEB.pdf