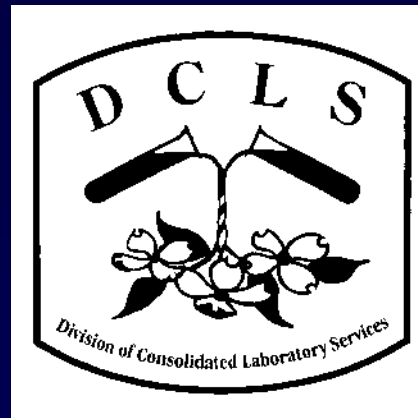


# Laboratory Response in the Commonwealth of Virginia to the Intentional Release of *Bacillus anthracis*



**Denise A. Pettit**

**J.V. Carroll, E.M. Basinger, M.R. Ettinger, S.S. Walker,  
T.L. York, and J.L. Pearson  
Commonwealth of Virginia  
Division of Consolidated Laboratory Services**



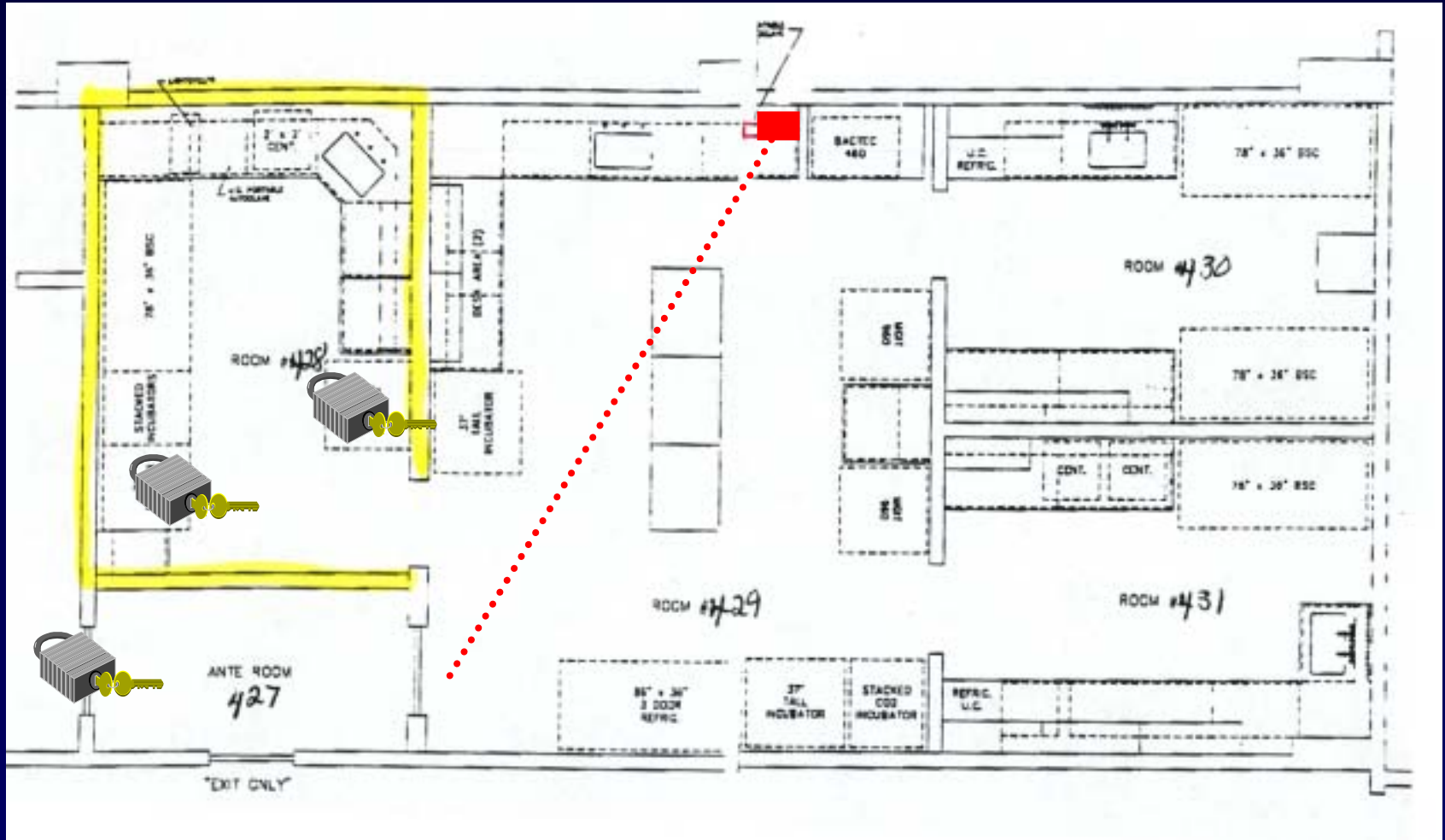
# Virginia Preparations

- **1999 Public Health Preparedness and Response to Bioterrorism Award**

**To enhance laboratory capacity for the detection of biological and chemical agents associated with terrorism**



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- **Activities initiated**

- **BSL 3 laboratory renovation**

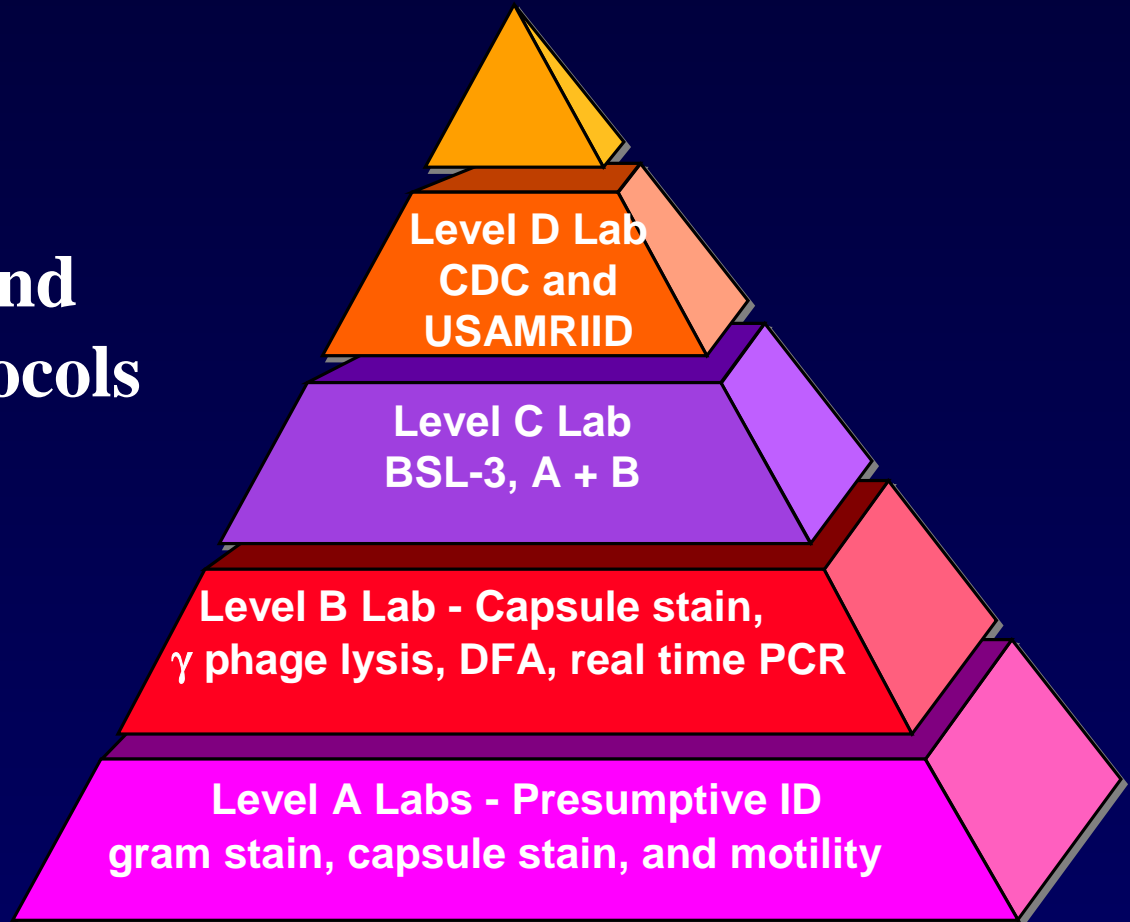
- **Establishment of the Laboratory Response Network in Virginia**

- **Provided Level A laboratory training**
- **Received Level B laboratory training**



# Laboratory Response Network

- Implementation and validation of protocols
- Reagents





# Virginia Preparations

- **1999 Public Health Preparedness and Response to Bioterrorism Award**
  - To enhance laboratory capacity for the detection of biological and chemical agents associated with terrorism
- **Activities initiated**
  - BSL 3 laboratory renovation
  - Establishment of the Laboratory Response Network in Virginia
  - Built partnerships with local, state, and federal agencies



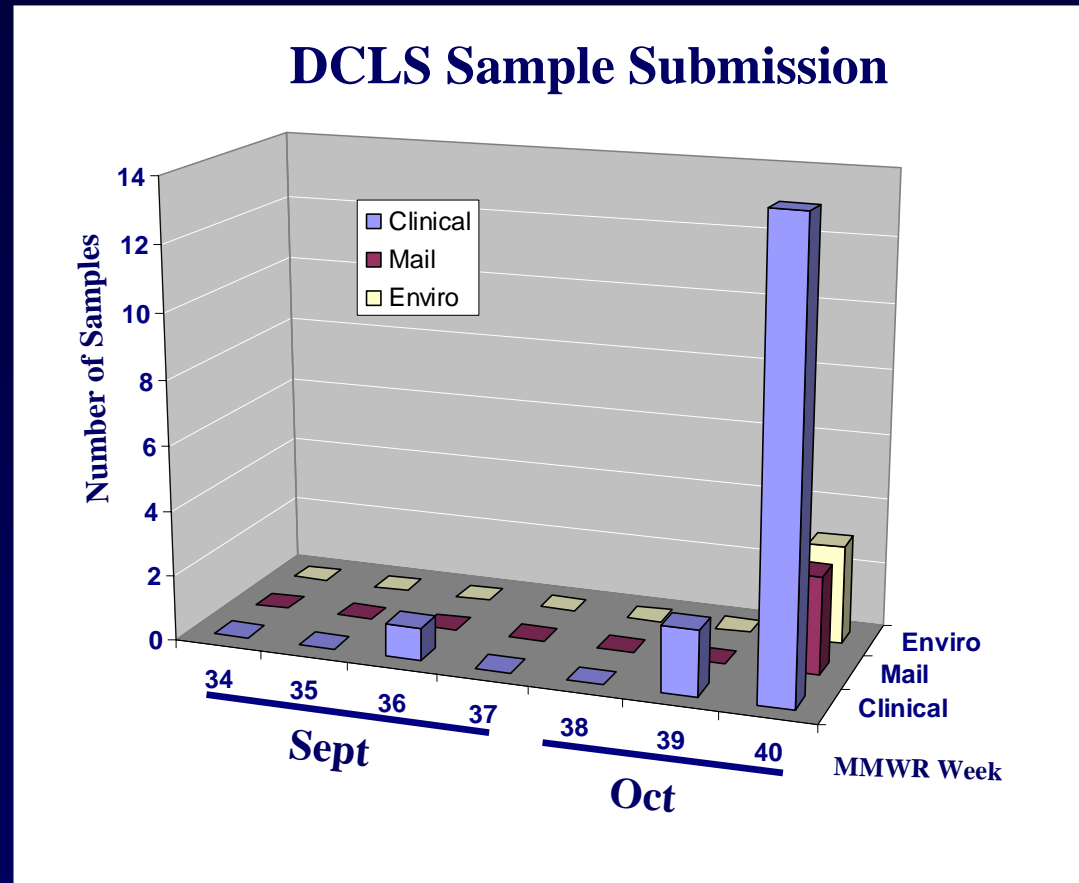
# Collaboration

- **Federal**
  - CDC
  - FBI
  - DOD
  - USAMRIID
  - DARPA
  - USDA
  - FDA
  - EPA
  - DOJ
- **Local/State**
  - Law Enforcement
  - HAZMAT
  - AG's Office
  - Environmental Crimes Task Force
  - FBI
  - Fire Departments
  - DEQ
  - VDACS
  - VDH
  - National Guard



# The Test of Preparedness

- October 4th (wk 39)
  - Florida resident
  - Inhalation anthrax
- October 9th (wk 40)
  - New York resident
  - Cutaneous anthrax



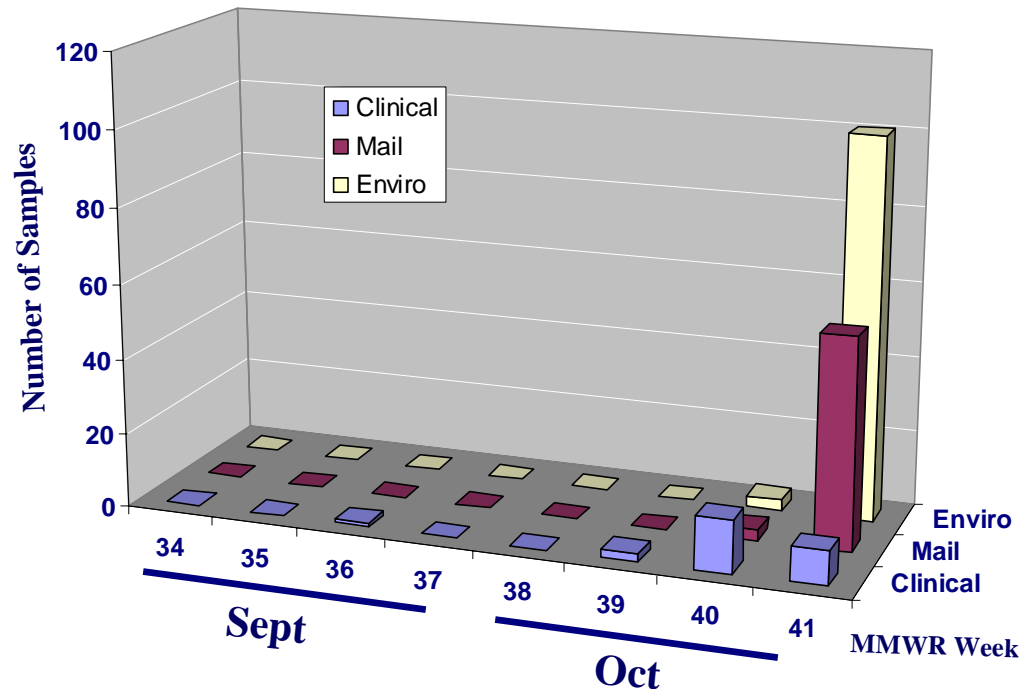




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## DCLS Sample Submission





# The Test of Preparedness



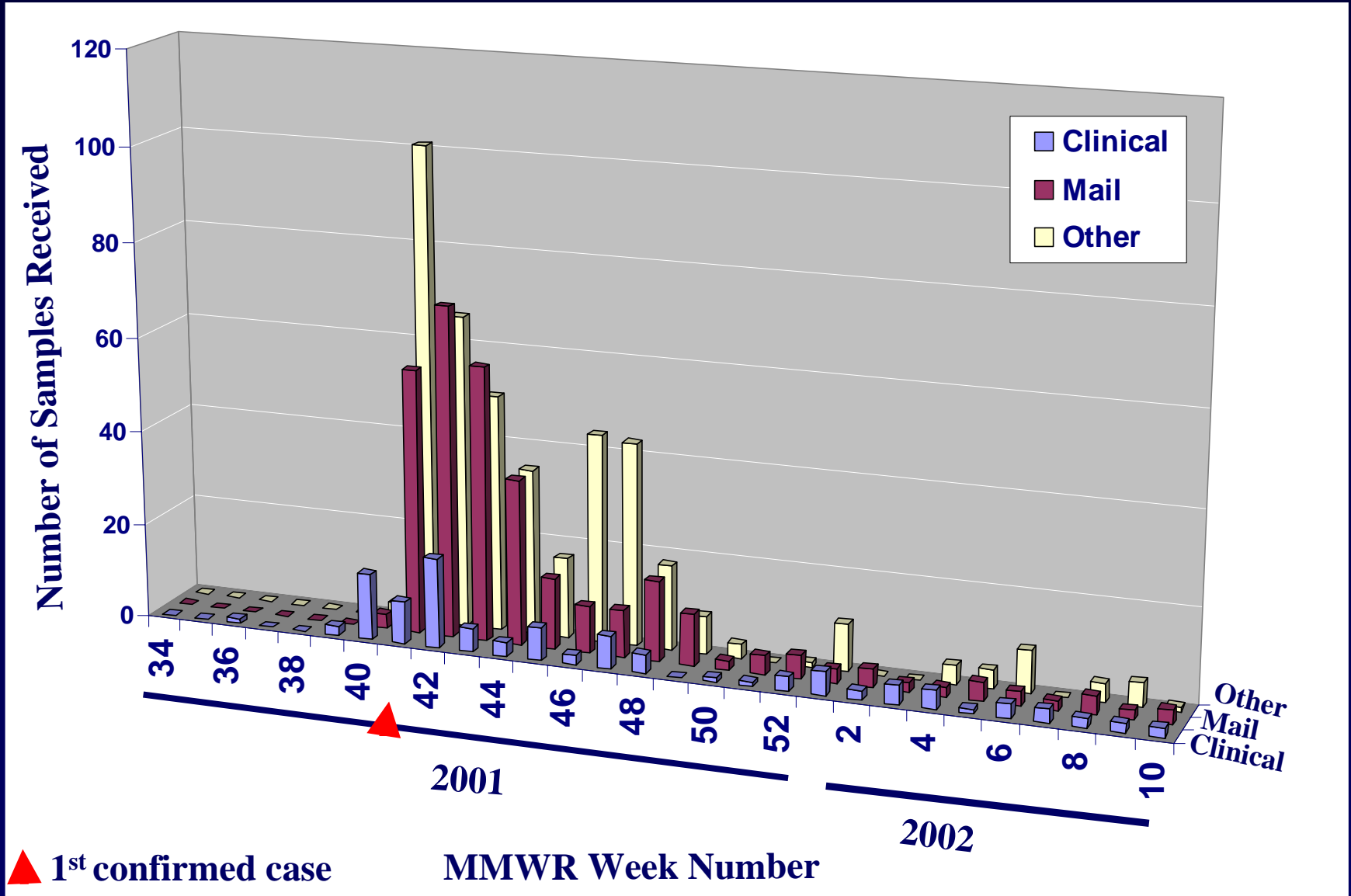
*Bacillus anthracis*  
Vegetative cells

- October 16th
  - Letter to U.S. Senate office confirmed positive
- October 19th
  - Enhanced regional surveillance activity identifies a suspicious patient
- October 20th
  - Level A presumptive positive
  - Level C confirmed positive



# Sample Submission

*B. anthracis*





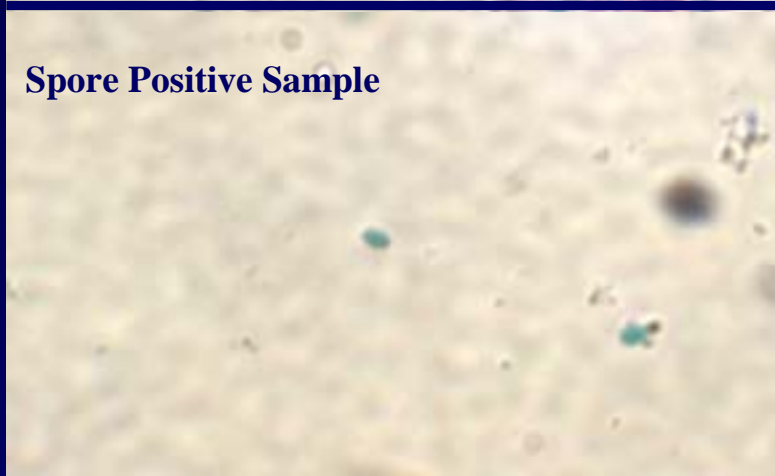
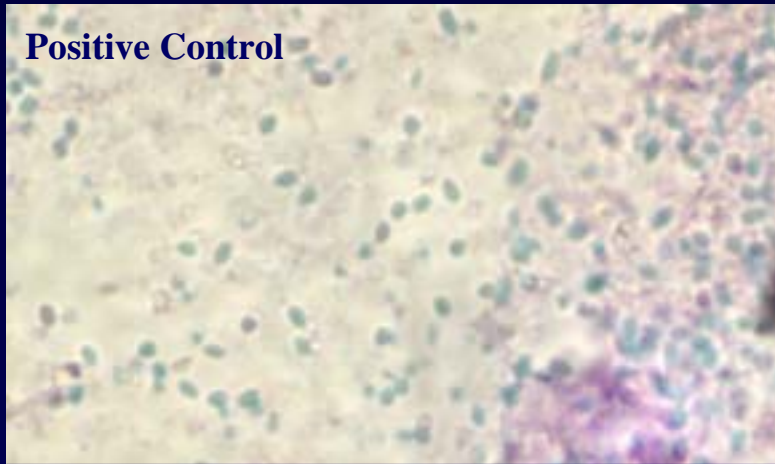
# Clinical Isolate Analysis

- 106 Clinical specimens
  - 66 isolates from Level A labs
    - 3 *B. anthracis* confirmed
    - 19 *B. cereus*
    - 15 No *Bacillus* sp. recovered or unable to speciate
    - 9 *B. subtilis*
    - 9 *B. brevis*
    - 4 *B. megatarium*
    - 3 *B. licheniformis*
    - 2 *B. circulans*
    - 2 *B. firmus*
  - 40 Specimens with NSG





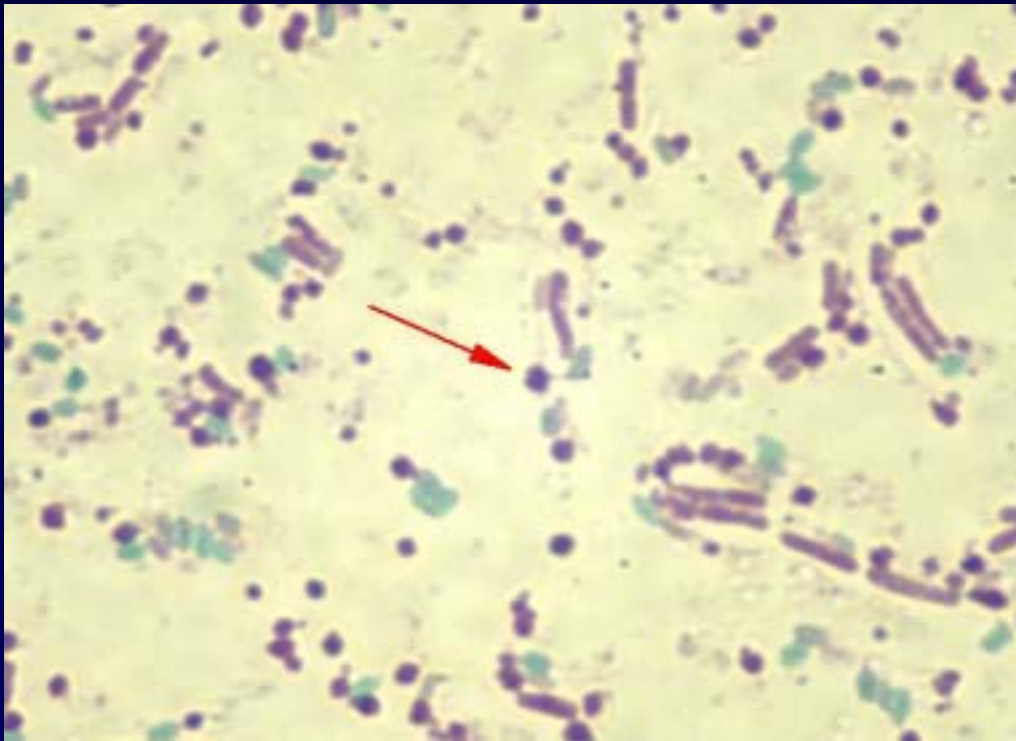
# Analysis of Mail



- **323 items analyzed**
  - 317 No spores observed
  - 6 Spores detected
- **Spore characterization**
  - Real time PCR negative
  - Culture
    - GPR
    - Beta-hemolytic
    - Motile
    - *B. cereus* group



## *B. cereus* vs. *B. thuringiensis*



*Bacillus thuringiensis*  
Spores and parasporal crystals

- Parasporal crystal in *B. thuringiensis*
- Malachite green modification
  - Heat fix smear
  - Flood with 5% malachite green, steam
  - Rinse
  - Cover with 0.1% carbol fuchsin (30 sec)
  - Rinse



# Analysis of Environmentals

- **741 samples**
  - **560 NSG**
  - **181 GPR**
    - **60 Beta-hemolytic**
    - **121 Non-hemolytic** (morphology indistinguishable from anthrax)
      - **40 Motile**
      - **81 Non-motile**
        - » **Negative gamma phage**
        - » **Negative DFA**
        - » **Negative real time PCR**
        - » **Similar biochemical profiles**



# Laboratory Response

- **Protocols**
  - Sample submission
  - Shipping and packaging
  - Chain-of-custody
- **Sample receipt**
  - Intake interview
  - Triage (?)
  - Evidence custodian
  - Evidence rooms







# Laboratory Response

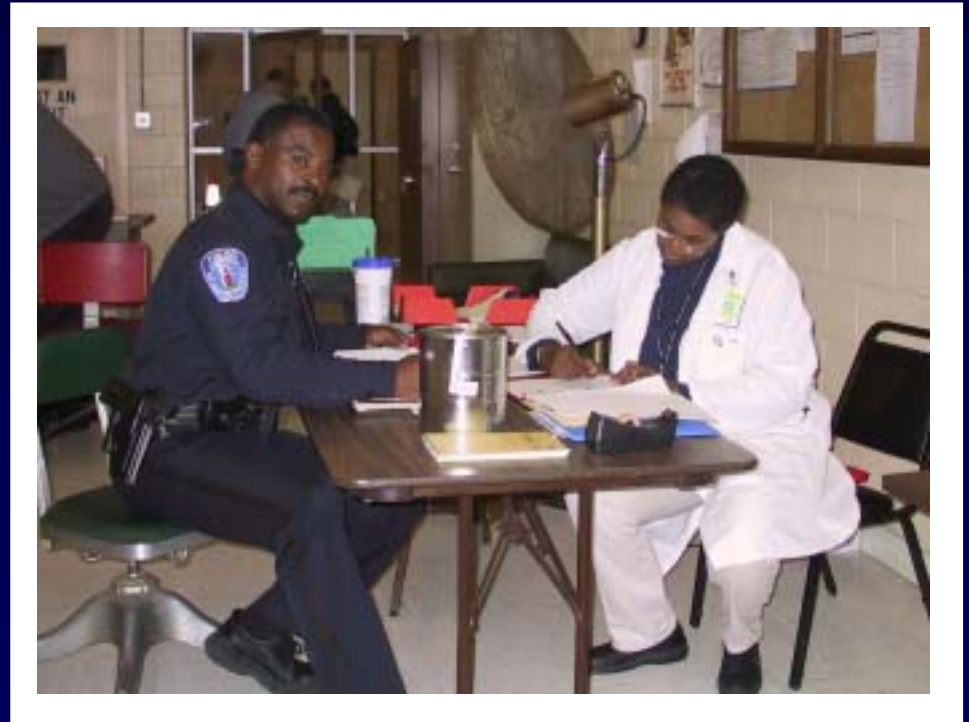
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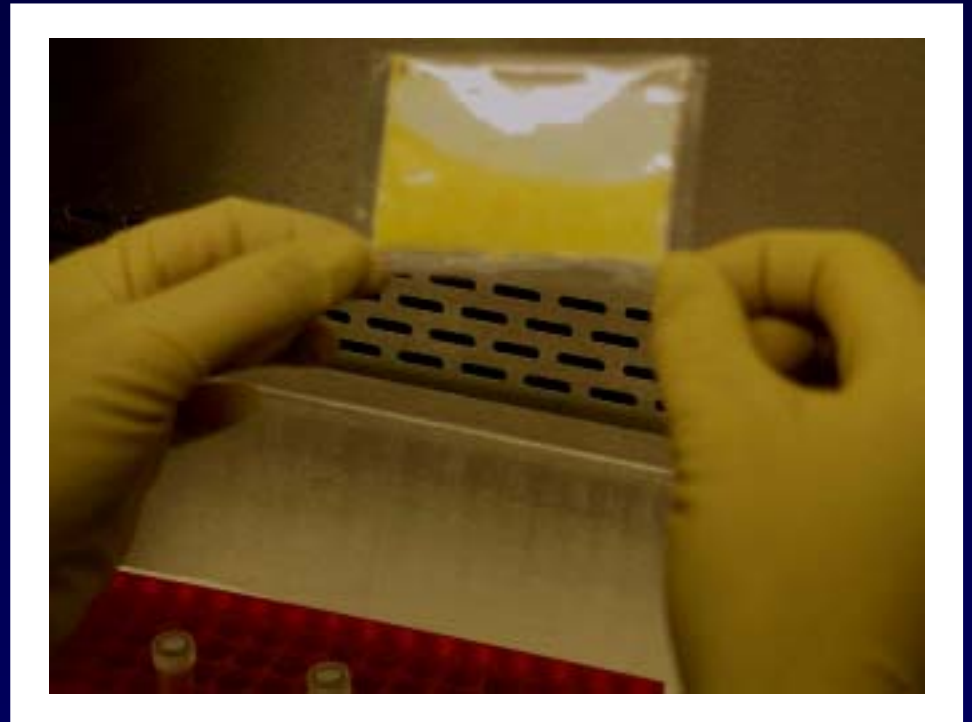
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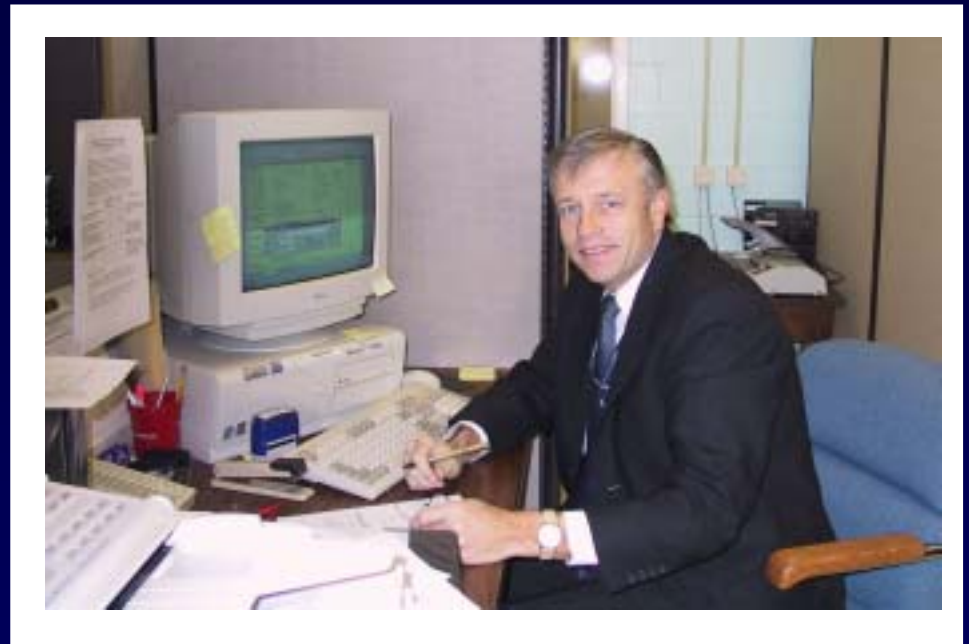
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# Laboratory Response

- **Communications**
  - Submitting agency
  - VDH
  - CDC
- **Train additional staff**
  - Management: emergency call
  - Scientists
- **Sample storage**
- **Evidence return**





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# Summary of the VA Experience

- Over 800 samples were processed to identify or confirm the presence or absence of *B. anthracis*.
- 3 of 66 clinical isolates submitted from Level A Laboratories were confirmed to be *B. anthracis*.
- *B. anthracis* spores were not identified in any environmental samples submitted.
- Parasporal crystals were identified in 8 environmental samples.
- Characterization of significant environmental isolates revealed no clinically significant *Bacillus* species.





# Acknowledgements

- **DCLS Staff**
- **Centers for Disease Control and Prevention**
- **Association of Public Health Laboratories**
- **Virginia Department of Health**
- **Local, State and Federal Partners**

**Dedication: June 2003**



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