Rapid Adaptation of a Serologic Assay for the Bioterrorism-related Anthrax Outbreak: October-December, 2001

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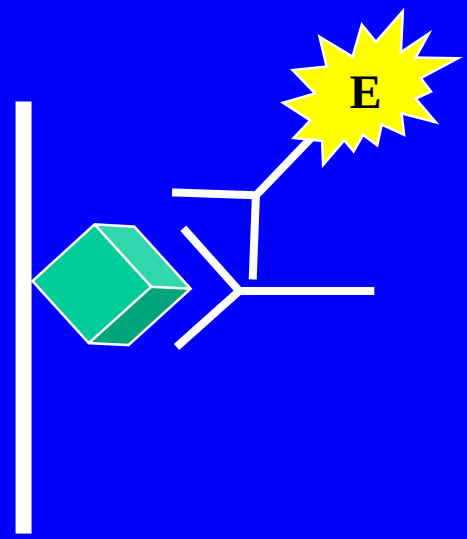
Outline

- The anthrax serologic assay and its adaptation

- Application to an emergency public health response:
  - Exposure: serosurvey in Florida
  - Mild disease: serosurvey in D.C.
  - Cases: identification and confirmation

Anthrax Serologic Assay in October 2001: Anti-PA IgG ELISA

- Developed for anthrax vaccine research program
- Immune response to protective antigen (PA) critical for protection
- Antigen: immobilized recombinant PA
- CDC human reference sera standard
- Not yet validated in outbreak/clinical setting



Adaptation of the Anthrax IgG ELISA, October 19-November 2

Rapid Validation (<2 weeks)

287 negative control sera: reactivity threshold (≥3.0 ug/ml)

230 sera non-anthrax infection: non-reactive

87 cohort of vaccinees/non-vaccinees: sensitivity/specificity

↓

Sensitivity: 98.0%

Specificity: 79.0%

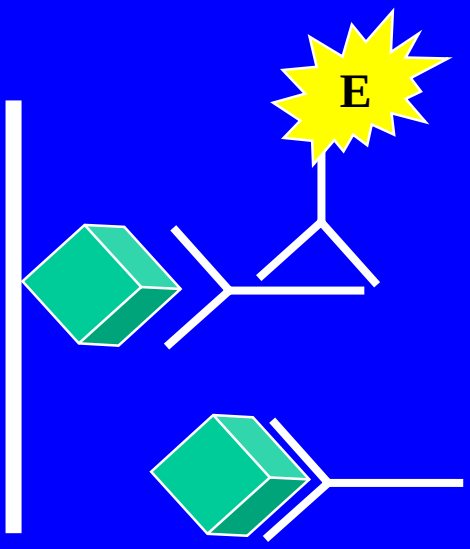
↓

Development of 2nd stage of assay to increase specificity

Competitive Anti-PA IgG ELISA

- Suppression of Ag-specific Binding -

- Sera with excess antigen compared to sera without excess antigen
- Calculation of percent inhibition of PA-specific binding
- ≥ 85% suppression for sera with anti-PA specific antibodies



Excess Ag prevents binding of specific antibody

Assessing Exposure: Florida Asymptomatic Serosurvey

- 430 exposed with paired sera tested
  - 4 with reactive 1st stage ELISA on both sera
    - None with 4-fold rise
    - None with 2nd stage competitive inhibition
- No evidence of anti-PA serologic response after exposure in the asymptomatic

A Florida Example

1st Serum Anti-PA Concentration	2nd Serum Anti-PA Concentration	Competitive ELISA % Inhibition
49.9 ug/ml	44.3 ug/ml	<30%

Excluding Mild Disease: Symptomatic D.C. Cohort

- Sera from 104 exposed, symptomatic individuals on post exposure prophylaxis
  - -51 paired sera
  - -34 acute sera only
  - -18 convalescent sera only
- 6 (5.8%) reactive in validated ELISA
- None demonstrated competitive inhibition
- No suggestion of mild anthrax disease by serology

Case Evaluation

Diagnostic Tests

- Blood culture
- Immunohistochemical staining (IHC)
- Polymerase Chain Reaction (PCR)
- Anti-PA IgG ELISA

Limitations

- Early antibiotic use
- No tissue available

A Serologic Scenario: New Jersey Case #2

9/18: Letters containing *B. Anthracis* postmarked

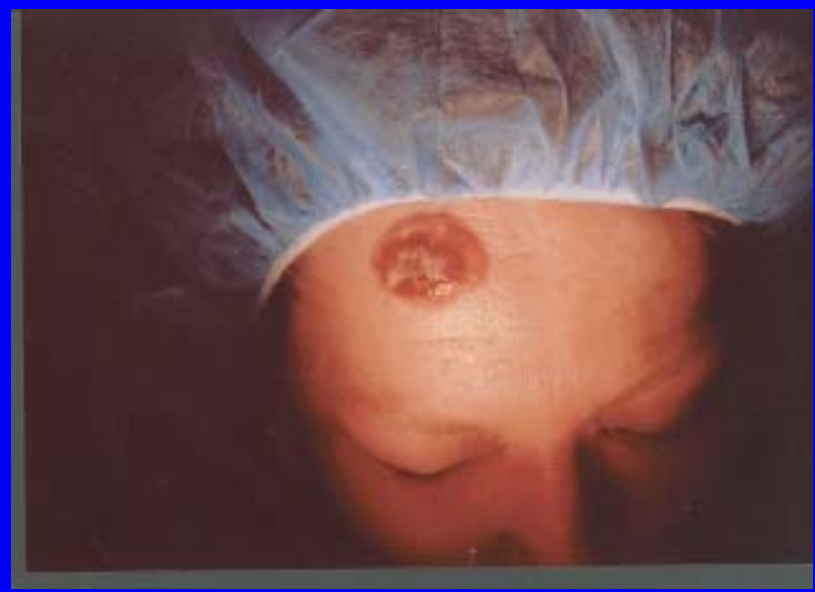
9/26: 39yo machine mechanic developed lesion

10/1: Antibiotics initiated (presumed cellulitis)

10/15: Evaluation for possible anthrax
Serum drawn → 163. 4 ug/ml
Competitive Inhibition → 85%

Evaluating Cases: Serology Confirms Cutaneous Cases

- 6/6 (100%) confirmed cases with specific reactivity
- 3/4 (75%) suspect cases with specific reactivity
- Serology confirmed 3/7 cases
- Serology identified 3/4 suspect cases (only laboratory test)



Evaluating Cases: Inhalational

- None of acute sera showed seroreactivity
  - 2-10 days post-symptom onset
- Earliest rise in anti-PA antibody concentration: 12 days post-symptom onset
- 6/6 convalescent sera showed specific seroreactivity
  - 4-fold rise in anti-PA antibody concentration
  - Competitive inhibition

Summary

Anthrax 2-stage ELISA serologic applied to:

1. **Exposure:** Asymptomatic individuals at risk did not develop antibody to PA
2. **Mild anthrax disease:** Individuals at risk and with flu-like symptoms on prophylactic antibiotics did not develop antibody to PA
3. **Possible cases:** Serology valuable in
    - Confirming cutaneous and inhalational cases
    - Identifying cutaneous cases when other tests not available

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