Laboratory Confirmation of Human Cases of Botulism

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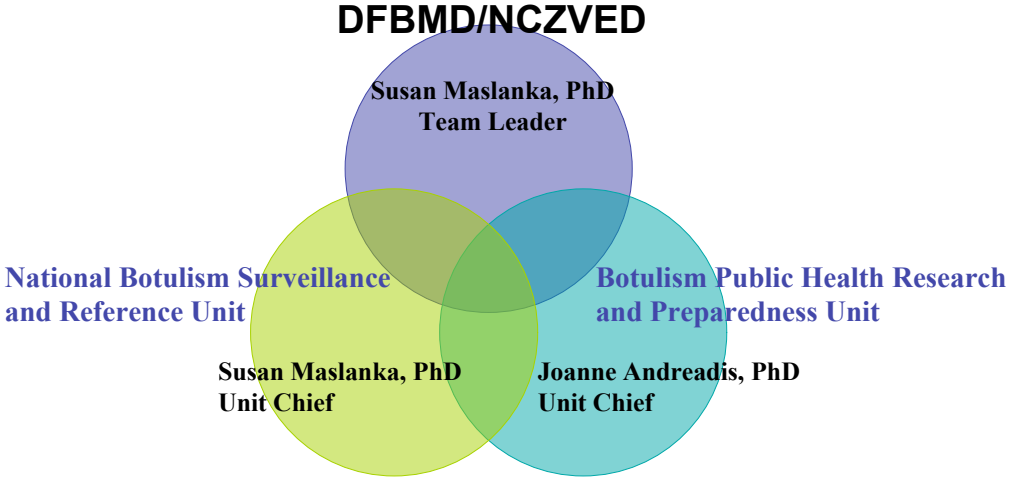

Maintaining US Scientific Expertise for Laboratory Confirmation of Botulism Through Outbreak Investigations and Research

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Botulinum toxin types

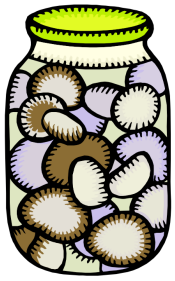
- Seven known toxins:A,B,C,D,E,F,G
  - Human botulism
    - A, B, E, F (C & D were described in late 1950's)
  - Animal
    - B, C, D, E
  - Natural disease unidentified
    - Type G

Neurotoxigenic *Clostridia*, sp

- *Clostridium botulinum*
- *C. baratii type F*
- *C. butyricum* type E

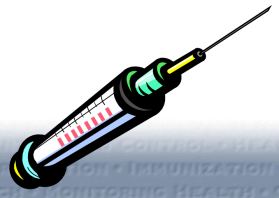
Botulism Types-

Foodborne

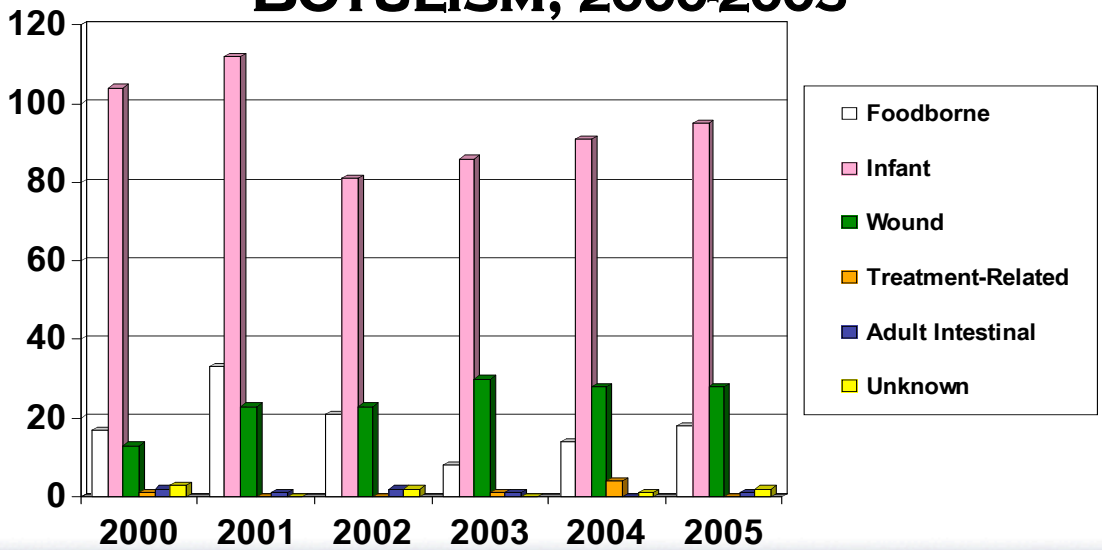


Infant
(& adult colonization)

Wound



U.S. Laboratory-Confirmed Botulism, 2000-2005



Global Disease

North America
USA & Canada

South America
Argentina & Brazil

Western Europe
UK, France & Italy

Central Europe
Poland & Germany

Eastern Europe
Russia & Ukraine

Southwestern Europe
Georgia & Spain

Southeastern Europe
Armenia & Romania

Prevalent on all continents

Northern Europe
Denmark & Finland

Southern Europe
Italy

Asia
China, Japan, Thailand

Middle East
Iran & Saudi Arabia

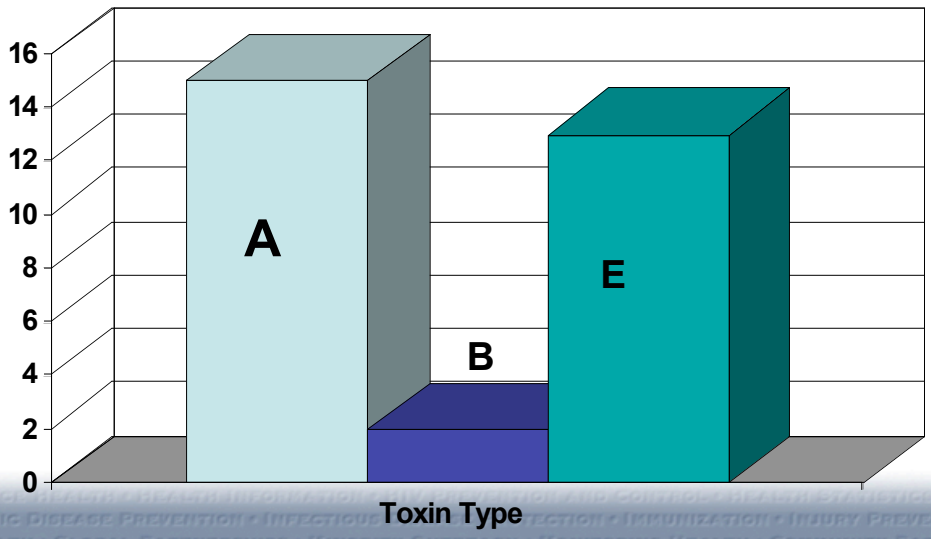
Africa
Egypt & Ethiopia

Oceania
Australia

Toxin Type of Foodborne Outbreaks

Region	Predominate Toxin type
North/South America	A (vegetables); E (fish)
Europe	B (meat in Germany, Belgium, Poland); B (vegetables in Italy, Spain)
Scandinavia	E (fish)
Soviet Union	E (fish); A (vegetables)
Asia	A (plant material); E (fish)

Fatality Rate



Acceptable Specimens

(from patients exhibiting symptoms consistent with the diagnosis of botulism, only)

Foodborne	Infant	Wound
serum, gastric, vomitus, stool, sterile water enema, food samples	serum, stool, rectal swabs, potential sources	serum, stool (in case not wound), tissue

All specimens should be maintained at 4 C, not frozen

Mouse Bioassay

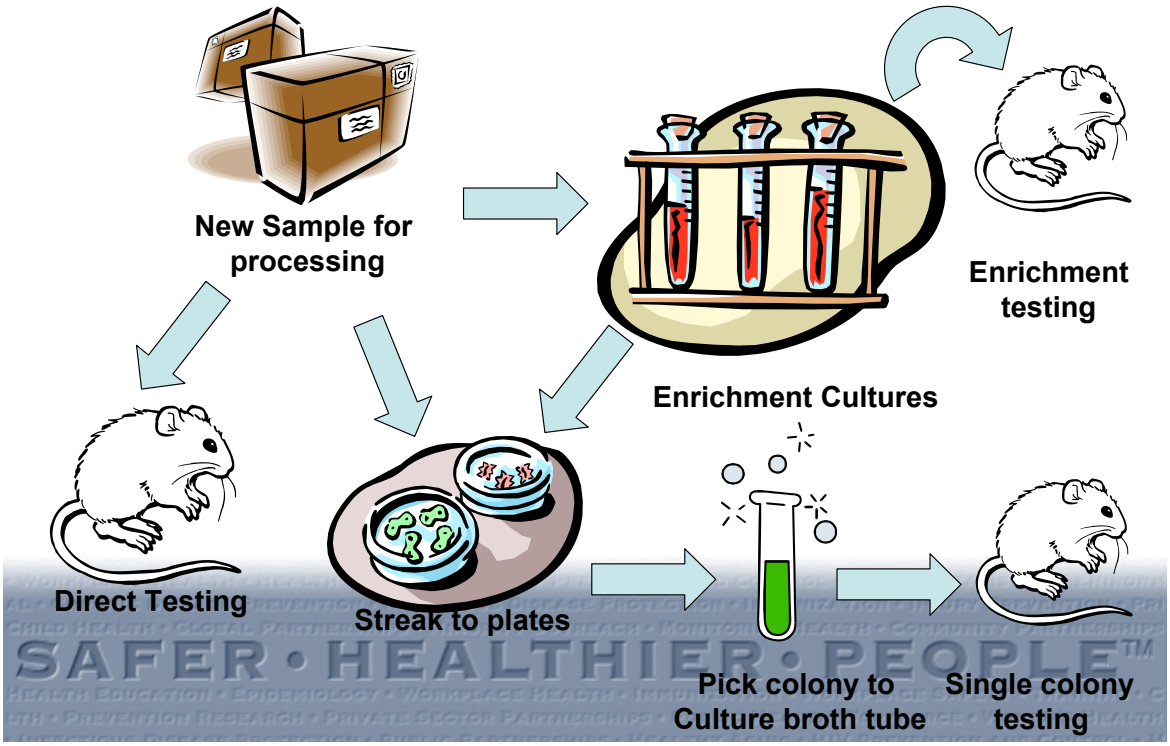
Advantages

- Exquisitely sensitive (10×10^{-12} grams= 0.000000000010 g)

Disadvantages

- Hazardous
- Extensive animal use
- Only 17 test sites around the country (14 state, 2 local, CDC)

Sample Processing for the Mouse Bioassay



Requirements for Toxin Potency in the Clinical Laboratory

- Determination of level of toxin in contaminated food source
- Determination of level of toxin in circulation of exposed patients
- Determination of level of toxin produced by isolated outbreak strain (s)

Impact on Animal Use

A single specimen

Botulinum Toxin ELISA
ABEF

- National testing capacity increased by 85%
- Time to detection reduced by 44%
- Sample throughput increased 67%
- Detects < 1 mouse lethal dose
- Test ruggedness: all reagents provided and quality controlled

The Botulinum Toxin Type A, B, E, or F ELISA Kits

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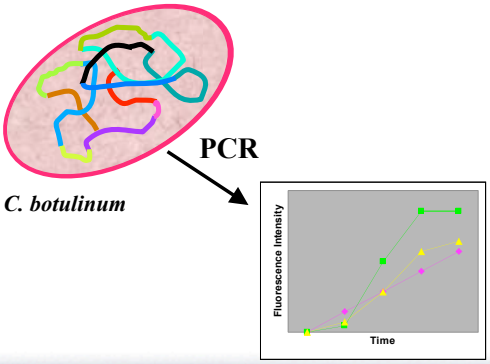
Rapid Toxin Detection and Identification

Real-time Polymerase Chain Reaction (PCR) (A-G)



Identification of toxin DNA in 0.3- 1.5 hr

Impact on Preparedness

- Laboratory testing algorithm streamlined
- Time-to-identification reduced 5-15 days
- Response within 1st 24 hrs improved
- Reduced animal use

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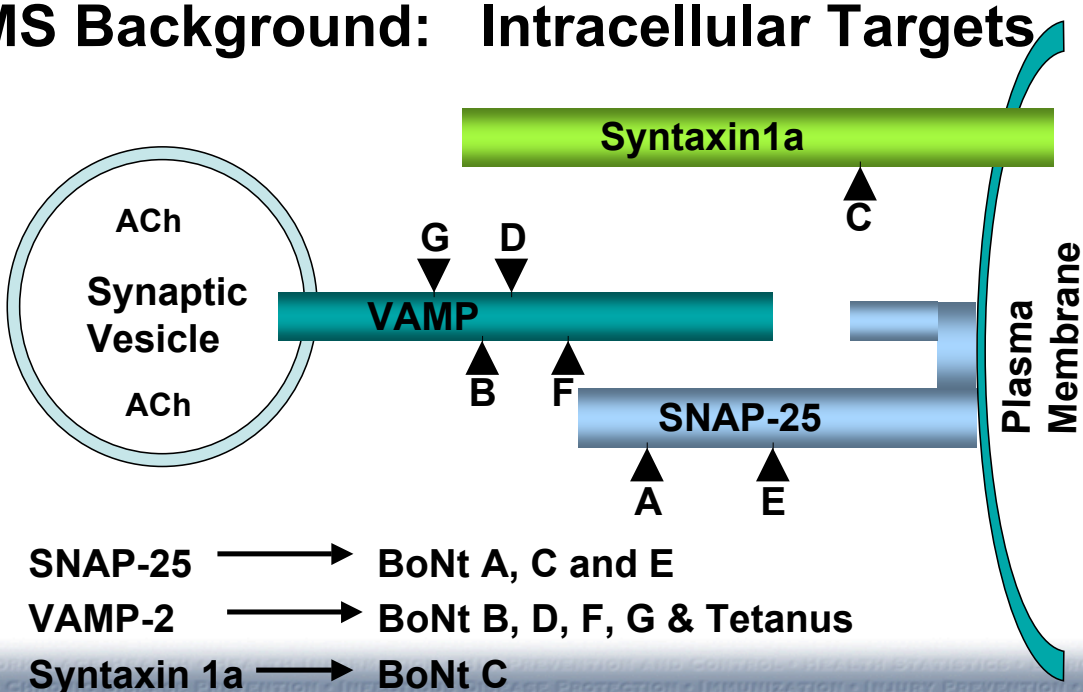
Rapid Detection and Identification of Organism

MS Background: Intracellular Targets



SNAP-25 → BoNt A, C and E

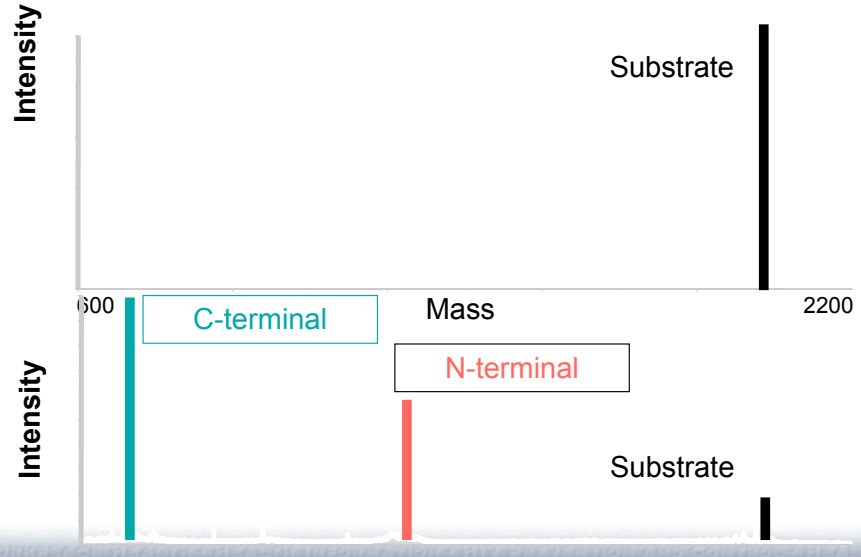
VAMP-2 → BoNt B, D, F, G & Tetanus

Syntaxin 1a → BoNt C

The seven different serotypes of BoNT target different SNARE proteins and/or protein sites.
Cleavage of any one of these proteins prevents complex formation needed for acetyl choline release.

MS Botulinum A cleavage products

Cartoon representation of test



Test	Strengths	Limitations
Mouse	Sensitive, active toxin, all toxin types, subtypes, and unidentified, can direct treatment	Limited US capacity, can take up to 4 days; nonspecific death occurs occasionally
ELISA	Sensitive, rapid (~4.5 hrs), can be deployed to many laboratories CDC ELISA detects all known subtypes of toxin A and B	Limited to 4 toxin types, may be insensitive to some toxin subtypes, may give false positives, matrix effects
MS	Sensitive, rapid (15 min to overnight), can be automated	Limited to known toxin types, may be insensitive to toxin subtypes, expensive, matrix effects unknown
PCR	Rapid Identification of organism	Cannot predict toxin production, matrix effects unknown

Challenges to In vitro Testing for Human Cases of Botulism

- 7 toxin types with unknown number of subtypes
- Numerous specimen matrices
- Low toxin levels in clinical specimens
- Regulatory issues for testing clinical specimens- in vitro device
- Regulatory issues for testing commercial products– FDA/USDA requirements
- Test availability