State of California

Department of Health Services

Prevention Services

Division of Communicable Disease Control

Microbial Diseases Laboratory Branch

David Jensen, Public Health Microbiologist II

Botulinum neurotoxin producing species of *Clostridium*

- *Clostridium botulinum*
- *C. baratii*
- *C butyricum*

Botulism

IF BOTULISM POISONING IS SUSPECTED—

NOTIFY YOUR

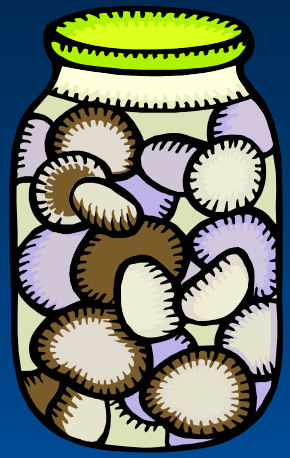
STATE PUBLIC HEALTH LABORATORY
OR
LOS ANGELES COUNTY PUBLIC HEALTH LABORATORY
(IF IN LOS ANGELES)

IMMEDIATELY FOR EVALUATION AND REFERRAL

Botulism Types

Intentional Contamination

Foodborne



Infant
(& adult colonization)

Wound

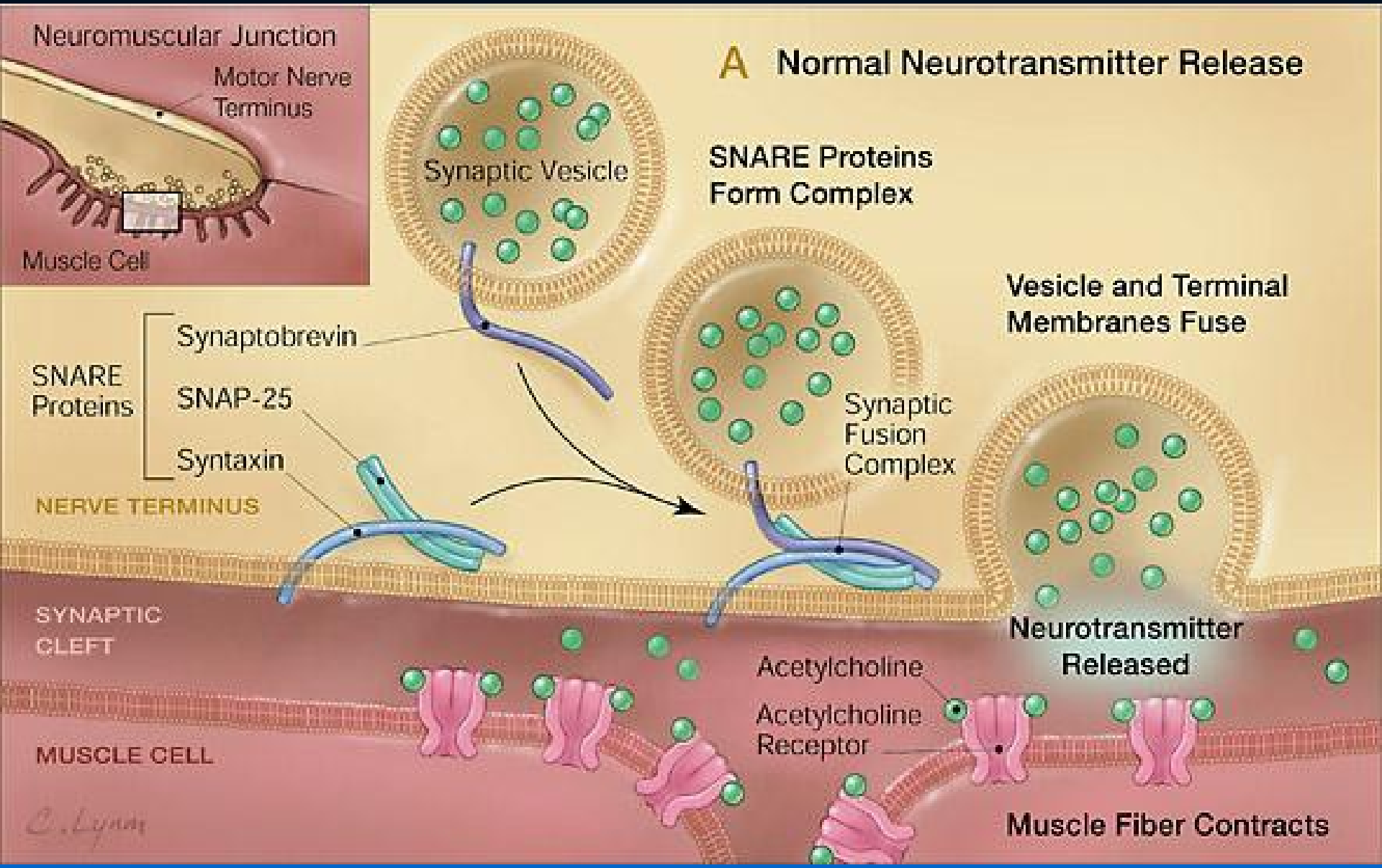
Botulinum toxin types

- 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

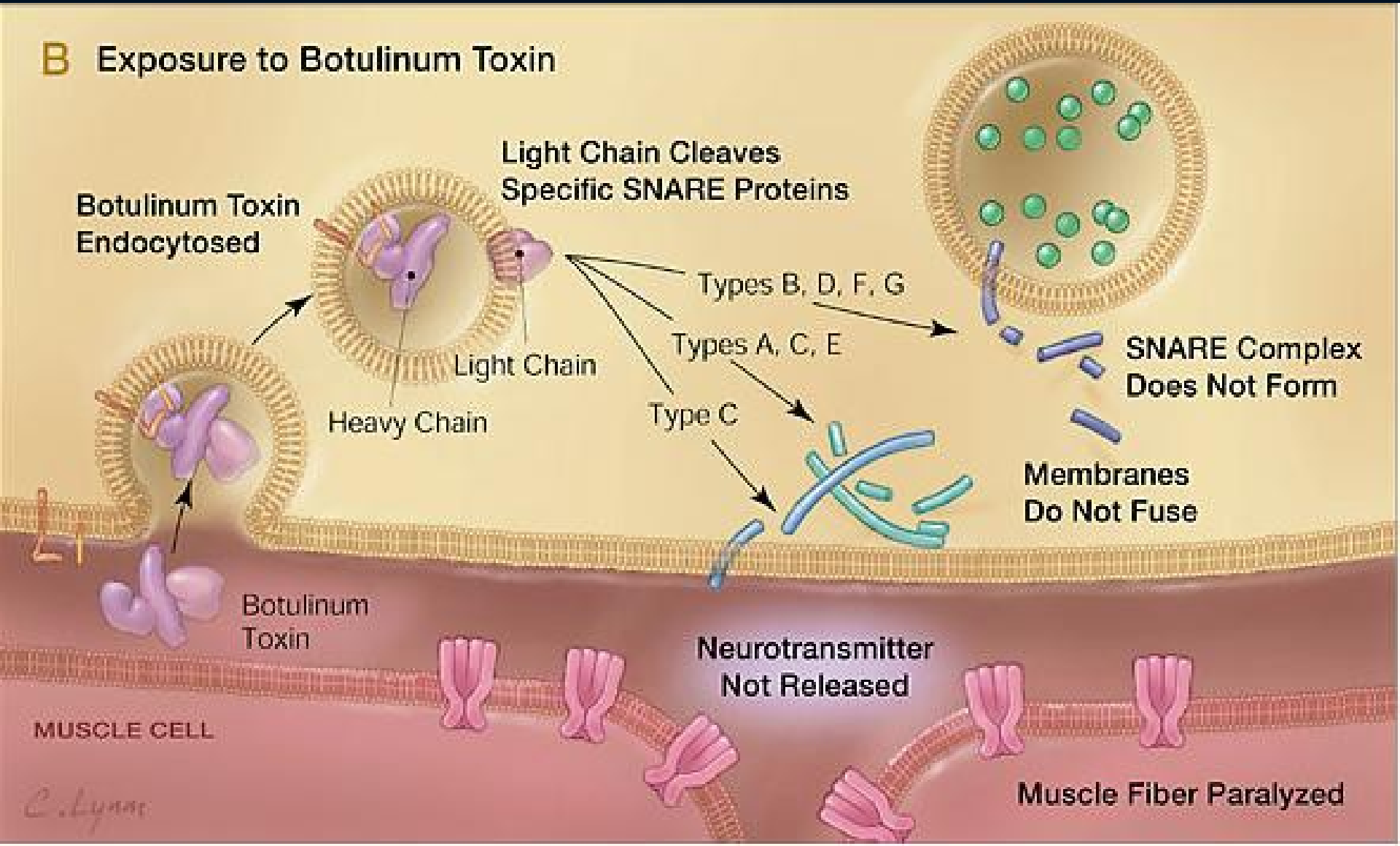
Botulism

- The preliminary diagnosis of botulism is made clinically, i.e., based on the patient's case history and physical finding
- Botulinum toxin, or botox, produces a neuroparalytic illness

Botulinum neurotoxin



Botulinum neurotoxin



Botulism

Sentinel (Level A) laboratory procedures are designed to ensure proper:

- Collection
- Packaging and shipping
- Safety

Level A Biosafety Alert

- Minute quantities acquired by ingestion, inhalation, or by absorption can cause profound intoxication and death
- Materials suspected of containing botulism toxin must be handled as BSL-2 specimen

Laboratory-acquired cases:

There has been one report of 3 cases of laboratory-associated inhalation botulism

Holzer E. Botulism Caused by Inhalation. *Med. Klinik*. 1962;No. 41:1735-1740.

Safe Work Practices

- Minute quantities of toxin are hazardous
- Decontamination
  - 0.1N sodium hydroxide
    - Will inactivate toxin
  - 10% household bleach (prepared fresh daily)
    - Will inactivate toxin
    - Will kill vegetative cells and spores
  - Treat spills sequentially (15 to 20 minutes each) with sodium hydroxide, bleach solution, and finally isopropyl alcohol (to reduce caustic effects of decontamination procedure)

Safe Work Practices

- Waste handling
  - All material with potential contact with botulinum toxin and/or *C. botulinum* must be autoclaved for 60 minutes, 121C, at 15 to 20 PSI
  - Since small quantities of toxin may cause illness, all material in the laboratory should be considered contaminated

Safe Work Practices

- Response to potential exposure
  - Workers should be made aware of early symptoms of botulism
    - Blurred or double vision
    - Dry mouth
    - Slurred speech
    - Peripheral muscle weakness
  - Self-monitor 2 to 4 days for symptoms
  - Report to emergency care facility if symptoms develop
  - Prophylactic antitoxin is not administered in the absence of symptoms

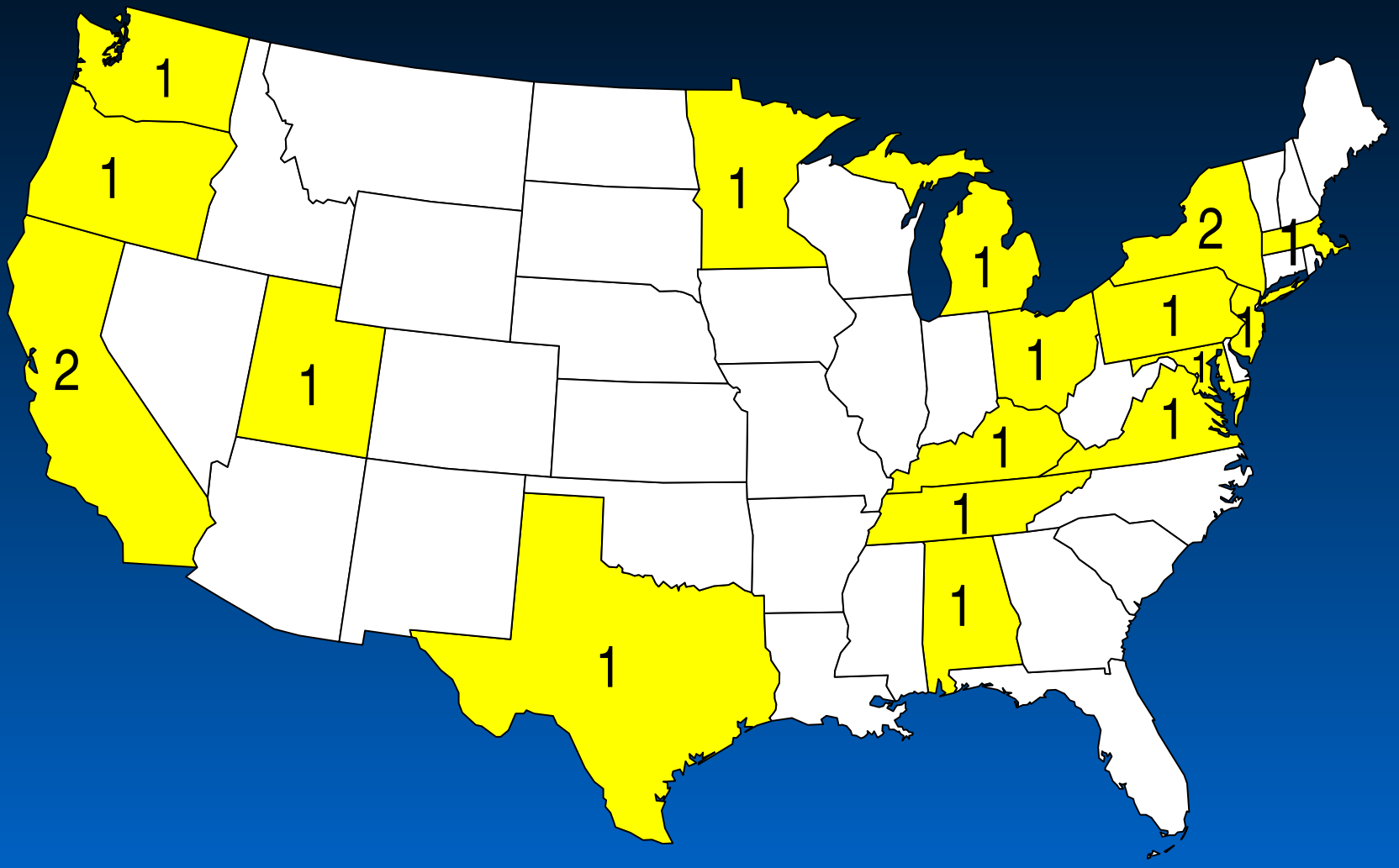
Botulinum Toxoid Vaccine

- "Investigational New Drug"- for the past 30+ years
- Available for laboratory workers through your health clinic after enrollment with CDC Drug Services (404 639-3356)
- Initial series: 0, 2 weeks, 12 weeks, 1 year
- Booster provided every 2 years following proof of need (serum submission to CDC for residual antitoxin test prior to boost)
- May eliminate future treatment options of therapeutic toxin preparations.

Botulism
MDL Specimens

1. Pre-antitoxin serum - no anticoagulant (~15mL)
2. Post-antitoxin serum – 6-12 h after antitoxin (~15mL)
3. Other:
    1. Stool-unpreserved (25g), Enema-sterile (25 mL)
    2. Gastric aspirate or vomitus (25 mL)
    3. Tissue or exudates (Port-A-Cul vials)
    4. Food, Soil (50-100g), Water (≥100 mL)

Laboratory Capacity for Botulinum Toxin Testing



Current approved test

- Mouse bioassay is the only currently approved test for the laboratory confirmation of botulism
- An ELISA (FDA/CDC) was recently validated for toxin detection in cultures
  - Currently under evaluation at CDC for utility in the clinical diagnostic laboratory

Mouse Bioassay

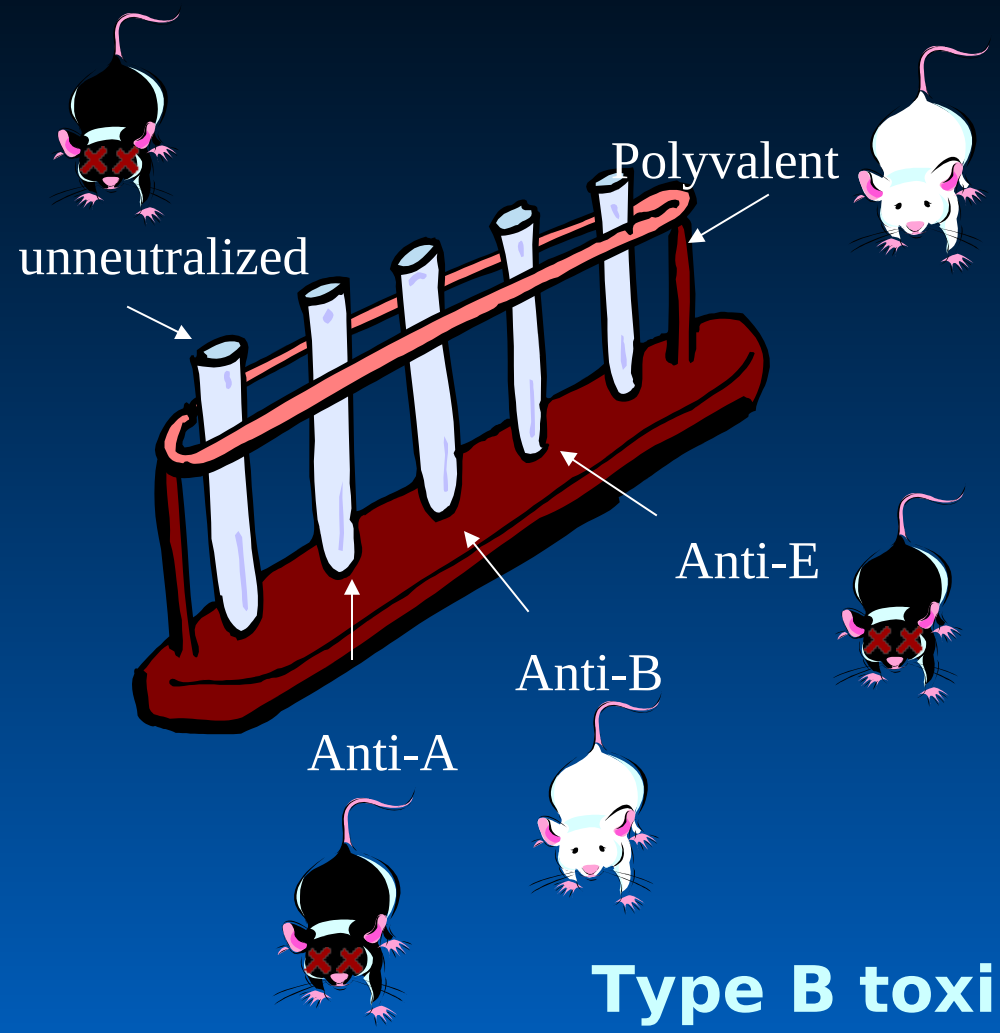
Advantages

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

Disadvantages

- Hazardous
- Extensive animal use
- Only 20 test sites around the country (17 state, 2 local, CDC)

Basis for Botulinum Toxin Detection



- Preparation of extract
- IP injection into 2 mice
  - Unneutralized
  - Neutralized with specific antitoxin
- 4 day monitor of mice for symptoms

Type B toxin identified

Botulism
Packaging and Shipping

- Consult your local public health lab for assistance
- Sterile leakproof container
- Insulated shipping containers with refrigerant (cold packs) 4-10°C
- Labeled “MEDICAL EMERGENCY, BIOLOGICAL HAZARD, REFRIGERATE ON ARRIVAL”
- Do not send USPS

Botulism
Packaging and Shipping

- Ship as hazardous materials
- Do not freeze or expose to heat
- Notify the laboratory receiving the specimens prior to shipment
- Forward patient history and clinical symptom information with specimens
  - Notify the lab of any medication that the patient is taking which may interfere with testing

REFER SPECIMENS TO THE

MICROBIAL DISEASES LABORATORY

(Richmond Campus)

OR

LOS ANGELES COUNTY PUBLIC HEALTH LABORARTORY

(if located in Los Angeles)

Your Laboratory Response Plan

- Develop and implement a bioterrorism response plan, test it, and update as needed
- Train your staff
- Know whom to call
- Know how and where to ship
- On advice of law enforcement —provide a chain of custody

Role of the Sentinel (Level A) Laboratory

Rule out critical biological agents

OR

Refer to higher level laboratory

QUESTIONS?