



Botulism Diagnostics: Challenges and Requirements

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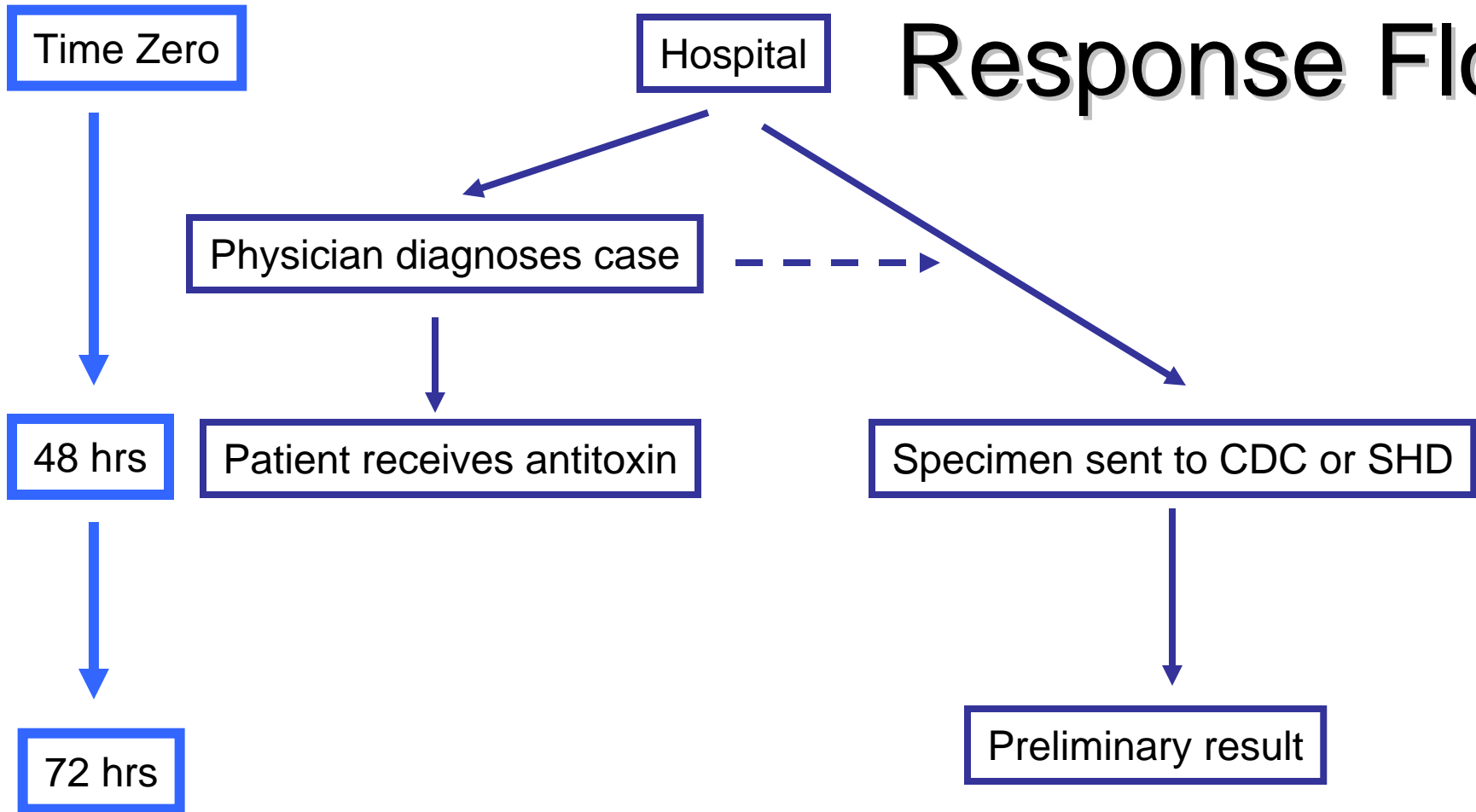


Current US Response

Algorithm for Diagnosis and Testing



Response Flow





Clinical Diagnosis

- Based on symptom presentation (classic triad)
 - symmetric, descending flaccid paralysis with prominent bulbar palsies
 - afebrile
 - clear sensorium
- Limited hospital tests
- Common misdiagnosis in single cases
 - myasthenia gravis
 - Guillain-Barré syndrome
 - stroke
- Multiple cases usually but not always correctly diagnosed
 - Home prepared sausage associated
 - Carrot Juice—Canadian cases, US FL case
 - Hot dog chili sauce



Laboratory Confirmation Criteria

- Clinical specimens
 - Direct toxin test positive **24 to 96 hrs**
 - Culture positive **6 to 10 days**
- Source specimens (food, etc)
 - Direct toxin test positive

Mouse bioassay is currently the only method to confirm human botulism cases

Treatment decisions MUST not wait for toxin test results

Time to collect/ship/receive specimens may take several days



Mouse Bioassay

Whole organism detection

Reflects true toxicity- signs of botulism observed
Incorporates both heavy and light chain functions

Sensitive (15 pg/ml dichain to 250 pg/ml complex)

Detects all known toxin types and subtypes

Limitations: limited US capacity, requires

Only test that has potential to detect unknown types- Type X
animals, hazardous procedure, detection

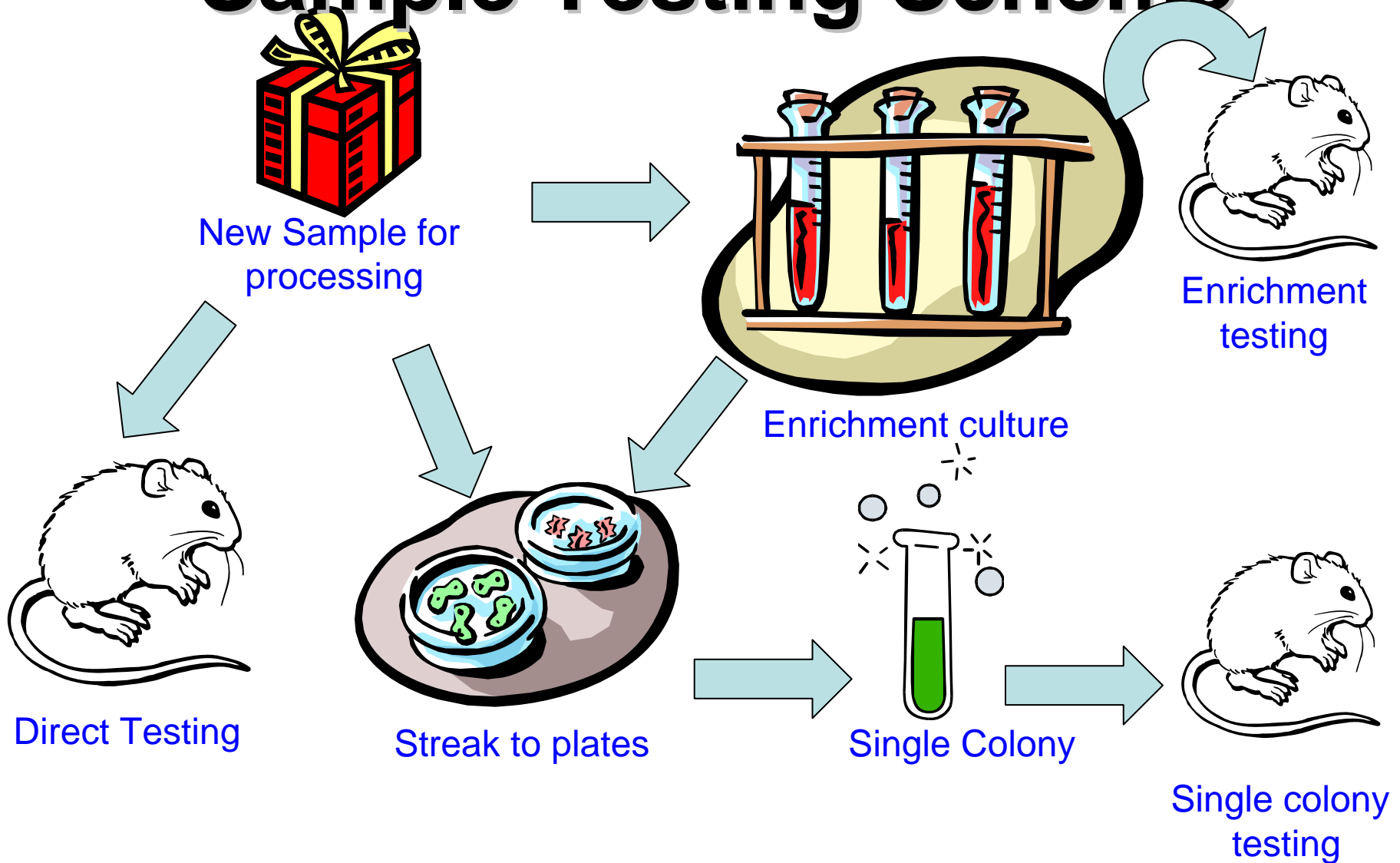
of low toxin levels may take 2 to 4 days

Historical precedent- used for over 40 years





Sample Testing Scheme





Purpose of Laboratory Testing

- **Natural event involving few cases**
 - Confirm cases identified by physicians
 - Identify outbreak toxin type to ensure appropriate treatment
 - Identify source of outbreak
- **Large suspect BT event**
 - Confirm cases identified by physicians
 - Identify and monitor outbreak toxin type
 - Identify source of outbreak
 - Screen large numbers of individuals with minimal symptoms, such as blurry vision
 - Screen large number of exposed individuals



In Vitro Diagnostic

Challenges to Development



Assay Target

Diversity

- Toxin serotypes
 - 7 known; A through G
- Recognition of subtypes
 - 22 reported
- Dichain vs complex
 - Mol wt 150 kDa to 900 kDa
 - Matrix dependent

Matrix Toxin Level

- Food
 - 40 to 600,000 mLD₅₀/gm
- Culture
 - 10 to 1,000,000 mLD₅₀/gm
- Serum
 - <1 to 2,000 mLD₅₀/ml
- Stool
 - <1 to 10,000 mLD₅₀/gm

Estimated Matrix Target Concentrations

Non-serum: 35 pg/gm to 35 ug/gm; Using type A complex 35 pg/mLD₅₀
Serum: 15 pg/ml to 30 ng/ml; using type A dichain 15 pg/mLD₅₀



Requirements for a Point of Care (POC) Diagnostic

- Sensitivity equivalent to mouse bioassay– 15 pg/ml of serum
- Must detect all toxin types A through G and all subtypes, known and unknown
- Specificity– minimal to no false positives
- cGMP or QSR compliant production
- FDA approval
- Commercially available
- Must predict therapeutic antibody treatment
- Rapid—within a few hours of clinical presentation
- Easy to use with minimal requirements for training



Barriers to POC Device

- Botulism is rare; little incentive for commercial development except under government contract
- Would require FDA approved device—validation?
- Maintaining capacity at all health care facilities
 - Cost
 - Easy to use device with little specialized skills
- No known in vitro device available that can predict therapeutic antitoxin requirement with absolute certainty
- Use of device may negatively impact public health response to natural events



Alternative to POC

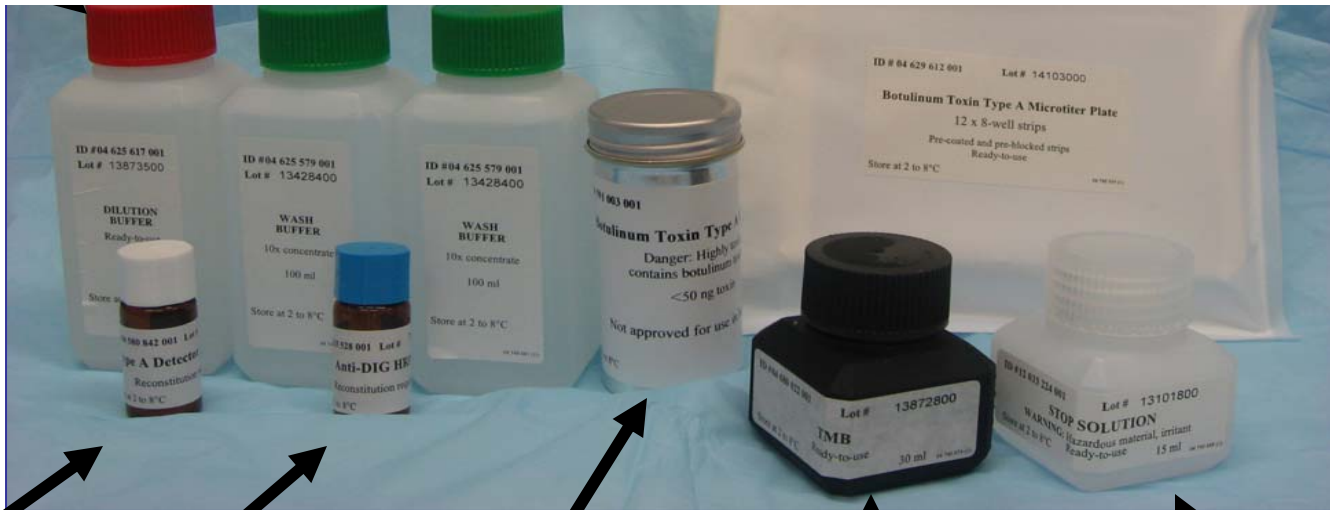
Enhance Public Health Capacity



Botulinum Toxin ELISA A, B, E, F



Distributed by Laboratory Response Network (LRN) and by Food Emergency Response Network (FERN)



Detector Ab

Amplifier

Positive Control

Substrate

Stop solution



Validation Approach

- **Limit of Detection (LOD)**
 - LOD dichain/complex in buffer
 - LOD dichain or complex in select matrices
- **Cross reactivity of pure toxin (dichain/complex)**
- **Inclusivity/exclusivity**
- **Matrix interference**
 - Food studies
 - Clinical specimens
 - Culture media
- **Case investigation samples-** in compliance with Human Subjects
- **Kit assessment**
 - Lot to lot variability
 - Stability
- **Enduser performance**
 - Detailed protocol/data interpretation
 - Training
 - Multilaboratory studies
 - Proficiency testing



BoNT ELISA Validation

Toxin subtype detection

Matrix	Limit of detection (ng/ml)			
	A	B	E	F
Buffer	0.02	0.10	0.04	0.20
Stool	0.06	0.09	0.06	0.20
Serum	0.03	0.06	0.03	0.15

Serotype	Subtype	Serotype Detected by ELISA			
		A	B	E	F
Type A	A1	+	-	-	-
	A1 (<i>ha-/orfX+</i>)	+	-	-	x
	A2	+	-	-	x
	A3	+	-	-	x
Type B	A(B)	+	-	-	x
	B1	-	+	-	-
	B2	-	+	-	-
	B3	-	+	-	-
Type E	Non-proteolytic B	-	+	-	-
	E1	-	-	+	-
	E2	-	-	+	-
	E3	-	-	+	-
Type F	<i>C. butyricum</i> E4	-	-	+	-
	Proteolytic F	-	-	-	+
	Non-proteolytic F	-	-	-	+
Bivalent	<i>C. baratii</i> F	-	-	-	+
	Ba (A4)	+	+	-	-
	Af (A2)	+	-	-	+
	Bf	-	+	-	+

	Direct Clinical (N=149)	Direct Food (N=41)	Enrichment Culture (N=433)	Pure Isolates (N=264)
Sensitivity	63%	100%	96%	99%
Specificity	99%	100%	93%	77%
Positive Predictive Value	95%	100%	82%	92%
Negative Predictive Value	91%	100%	98%	98%



Impact of BoNT ELISA

- Reduced the number of animals needed by 70%
- Decreased time for preliminary result from 24 to 6 hours
- Increased number of testing facilities (17 to 70)
- Increased sample testing capacity to 45,000 tests
- Harmonized testing method used by LRN and FERN
- Stable– 24 months; reduction of government costs
- Inter and intra- facility reproducible; facilitates data comparisons



Strengths/Limitations

- More rapid than mouse- 5 hrs
- Does not require animals
- Technology readily available
- Versatile strip well format
- Multiple Lot evaluations confirm lot to lot reproducibility
- Accepted by both LRN and FERN
- Provides surge capacity testing
- Kit performance defined through several years of studies
- Over 70 laboratories trained
- Contractor manufactured kits are reliable and stable
- Variety of specimen types: serum, stool, gastric, food, cultures, environmental powder samples
- Only detects 4 toxin types
- Antibody capture dependent so may be less sensitive to toxin subtypes
- Not currently approved in the US for clinical specimens so each lab must validate for in house use-
EUA In Progress
- Some foods known to cause some inhibition
- Expensive due to large minimum per lot
- Certain toxins bind to antibody on more than one kit
- May not predict toxin potency
- Reload time >4 months



Never Underestimate The Power Of a Mouse

**Only the mouse can tell us
what antitoxin will be effective**

Protection in mouse = Protection in patient



Challenges to Global Distribution of in vitro Methods

- Current products produced under US government contract according to US requirements; unable to predict Global need
- Mechanism to develop training and proficiency testing programs
- Distribution outside of LRN/FERN may impact US capacity and reveal vulnerabilities



Thank you for your attention

The findings and conclusions in this presentation are those of the author and do not necessarily represent the views of the Centers for Disease Control and Prevention