Project 4325-42000-049
Advance the Development of Technologies for Detecting and Determining the Bioavailability of Toxins that Impact Food Safety and Food Defense

Project Personnel: Luisa Cheng, Xiaohua He, Brad Hernlem, Reuven Rasooly, vice Stanker, vice Brandon

Luisa Cheng

Western Regional Research Center, Albany, CA

ARS/FSIS Shepherdstown, February 21, 2019

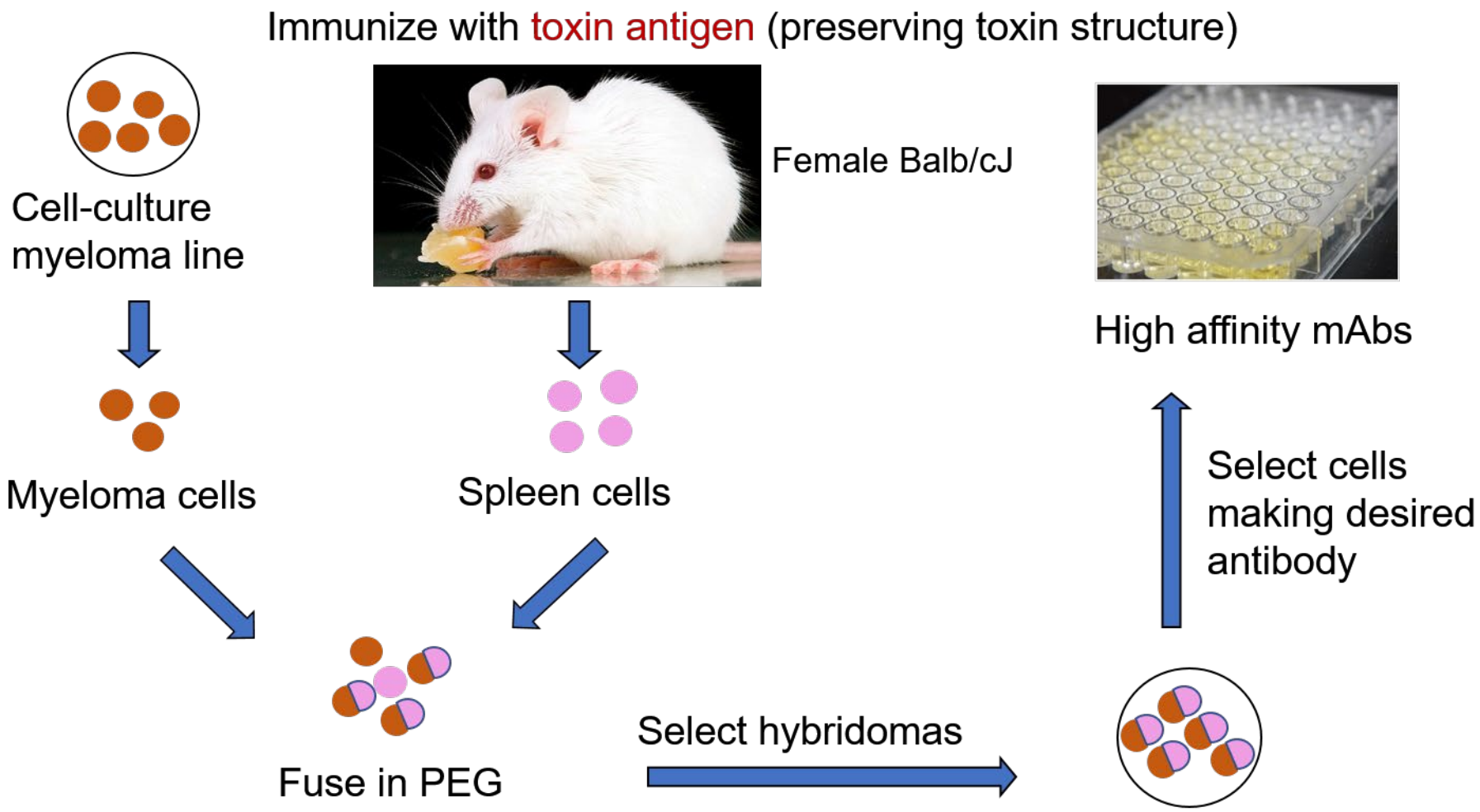
CRIS Objectives

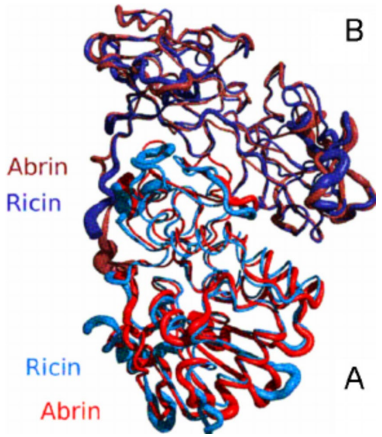
- Develop new antibodies (mAbs) to botulinum neurotoxins, toxin accessory proteins, shiga toxins, abrin, amatoxins. (Objectives 1, 2)

- Develop new detection assays (immunoassays, activity based, mass spec, new platforms) for Staph enterotoxins, abrin, BoNT, amatoxins and shiga toxins (Objectives 1, 2, 4)

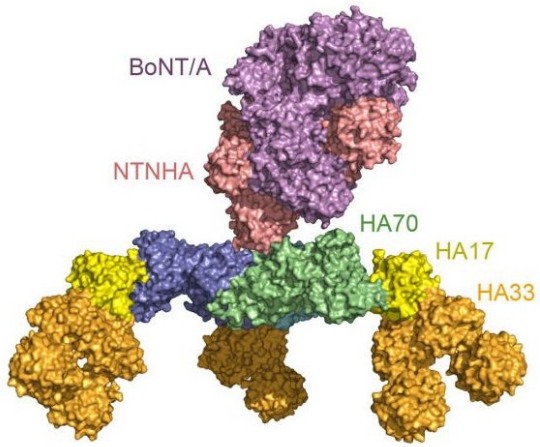
- Determine bioavailability of toxins in foods (pH, temperature processing, presence of foods or accessory proteins, other inhibitory molecules) with in vitro, and mouse bioassays (Objective 3)

Development of High Affinity Monoclonal Antibodies Against Toxins





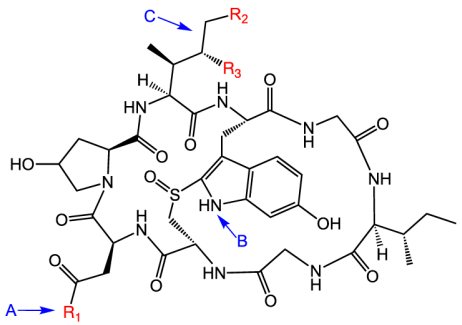
Toxin Antigen Types

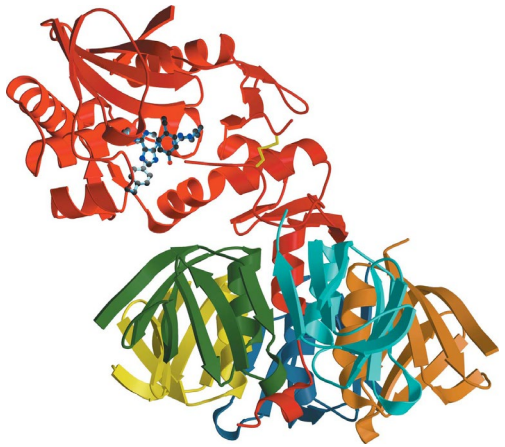


- Formaldehyde inactivated
- Heat inactivated (abrin)
- Iodoacetamide alkylation (botulinum neurotoxins)

- Recombinant toxins – Neurotoxin associated proteins (NAPs), catalytic domain mutations (shiga toxins)
- Recombinant toxin fragments (BoNTs)

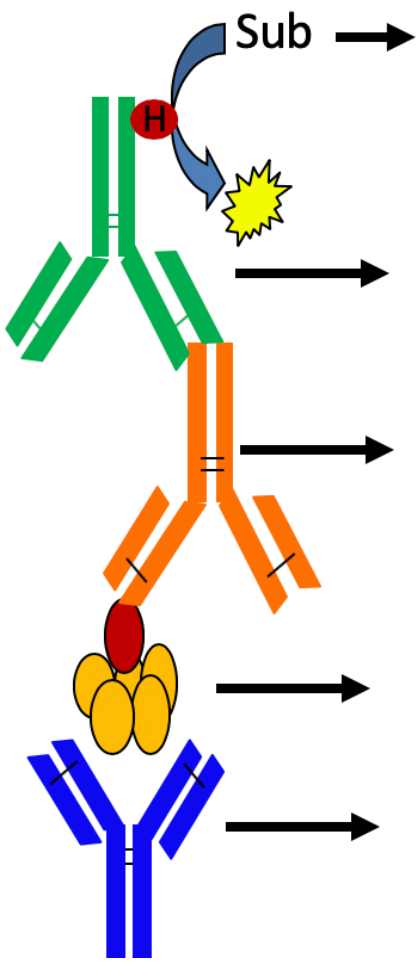
onjugation with protein linkers (amatoxins)





Strategies for Developing a Sandwich ELISA

- Screen antibodies for an EIA



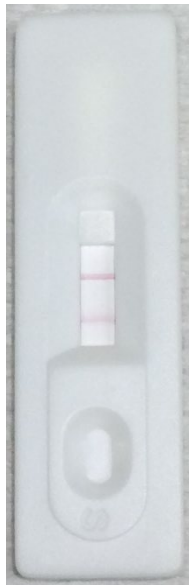
SuperSignal ELISA Pico Chemiluminescent

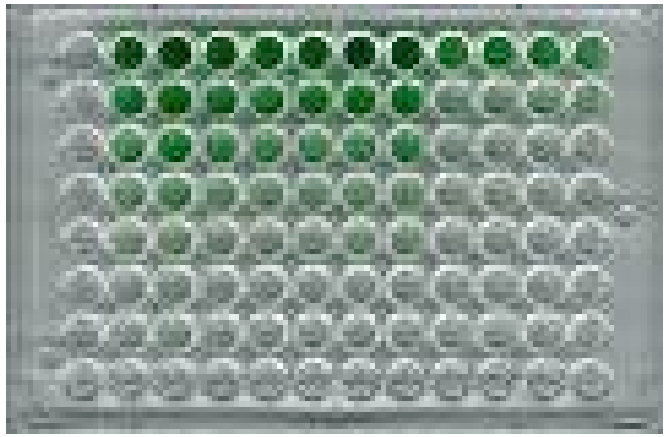
GAR-HRP

Detection antibodies: Stx1&Stx2-pAb

Stx1/Stx2

Capture antibodies: Stx1-2, Stx2-5, Stx2b-1
Stx2e-2, Stx2f-1





Challenges to the Development of Toxin Detection Assays

- Detect extremely low amounts for timely diagnosis and treatment

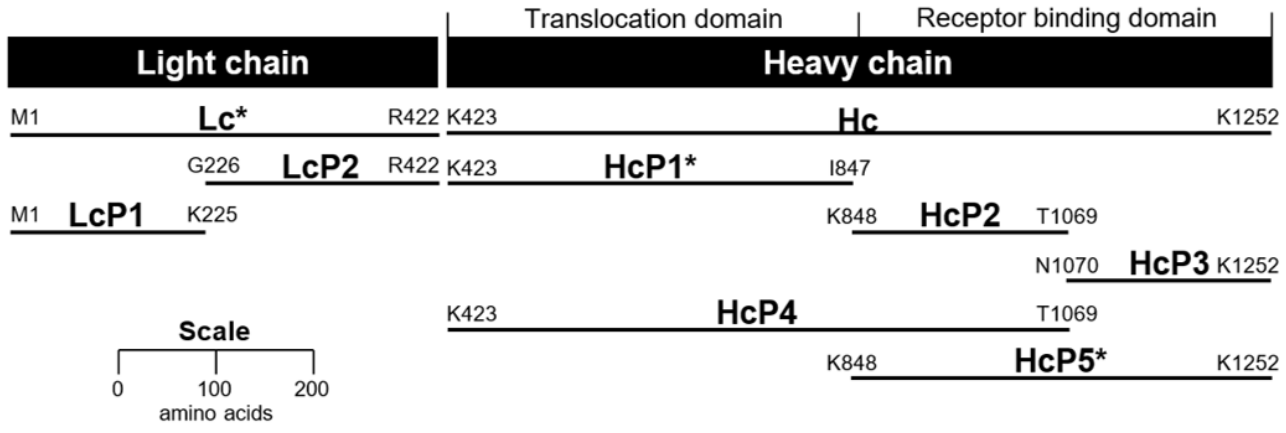
  Lethal oral dose of 70 µg botulinum neurotoxins (i.e.: Need LOD of < ng/ml in food?)

  IV dose of 20-200 ng (i.e.: Need LOD in pg/ml in sera?)
- Specificity: 8 serotypes (A-H), ~40 subtypes BoNT, 11 Shiga toxins
- Detection of active vs. non-active toxin
- Matrix effects: food, sera, feces, soil, etc.
- Ease of use
- Cost effective

Botulinum Neurotoxins

- Develop monoclonal antibodies to BoNT serotypes E, E NAPs, F, H
- Evaluate new assay formats e.g.: Pathsensors
- Determine bioavailability of BoNT in presence or absence of NAPs
- Determine bioavailability of BoNT +/- neutralizing mAbs, inhibitors, drugs

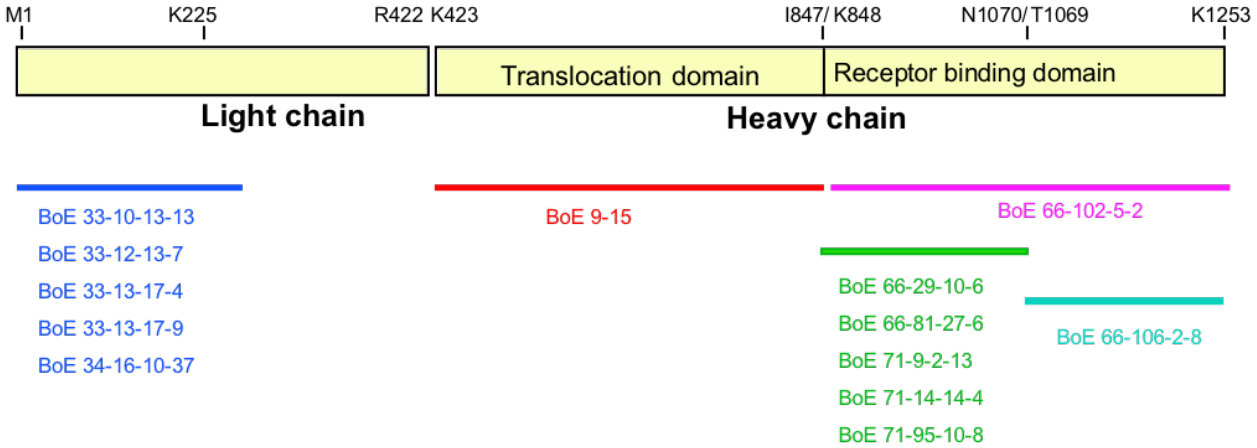
Development of antibodies to botulinum neurotoxin serotype E



* Indicates peptides used as immunogen.

Figure 1: BoNT/E peptide fragments. GST was cloned on the carboxyl end of each peptide.

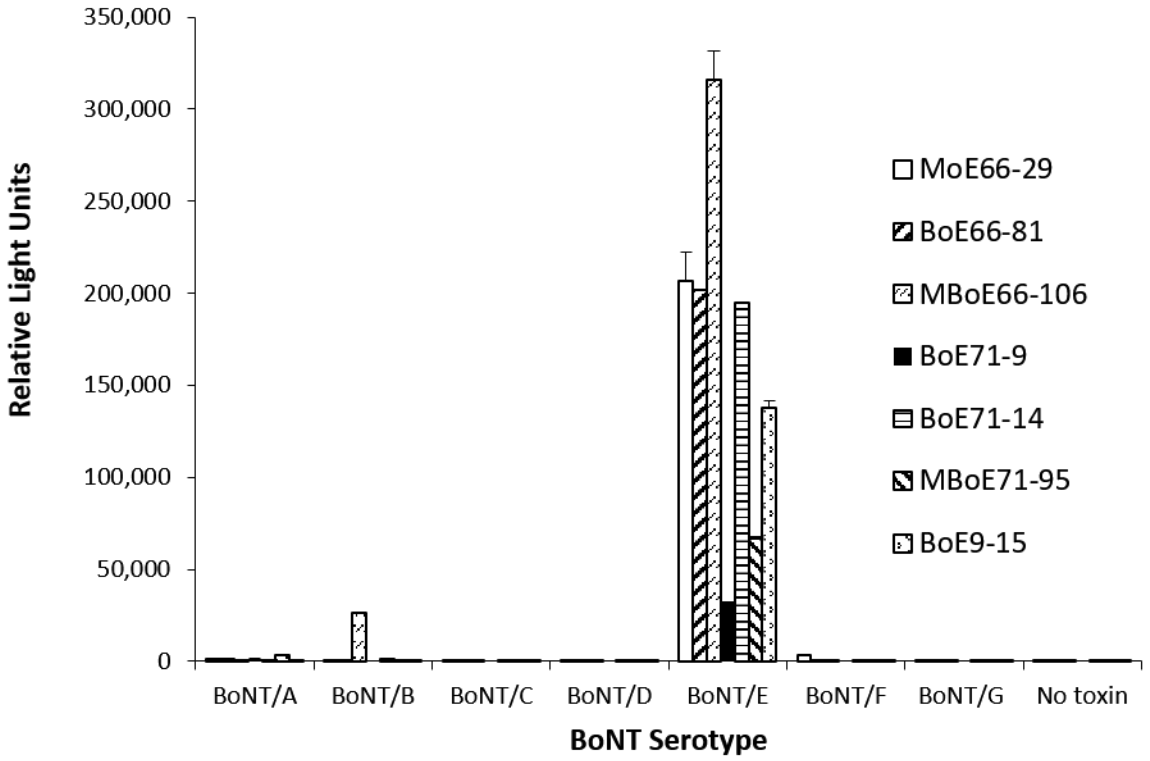
Figure 3: Epitope Identification of ant-BoNT/E monoclonal antibodies. Epitope location is based on antibody binding to recombinant toxin-GST fusion peptides. Diagram is drawn to approximate scale. The predicted transmembrane (K423 to I847) and receptor binding (K848 ro K1253) domains are indicated



Bever et al, 2019 Toxins, in press

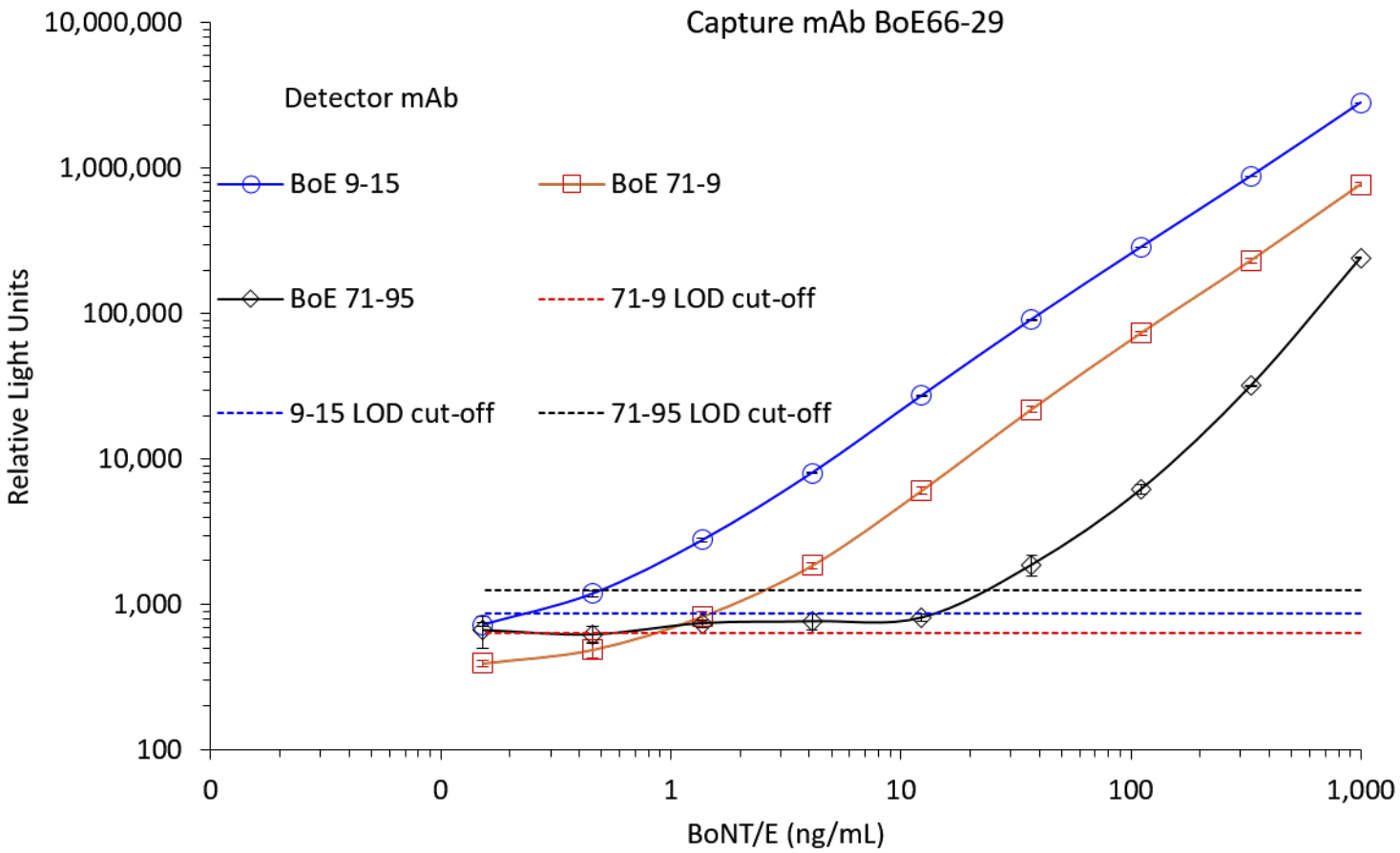
Selectivity of BoNT/E antibodies

Figure S2: Binding of Hc-Specific anti-BoNT/E mAbs to Toxin Serotypes



Bever et al, 2019 Toxins, in press

ELISA for BoNT/E



Limit of Detection (LOD):
~ 100 - 200 pg/ml

Bever et al, 2019 Toxins, in press

toxins

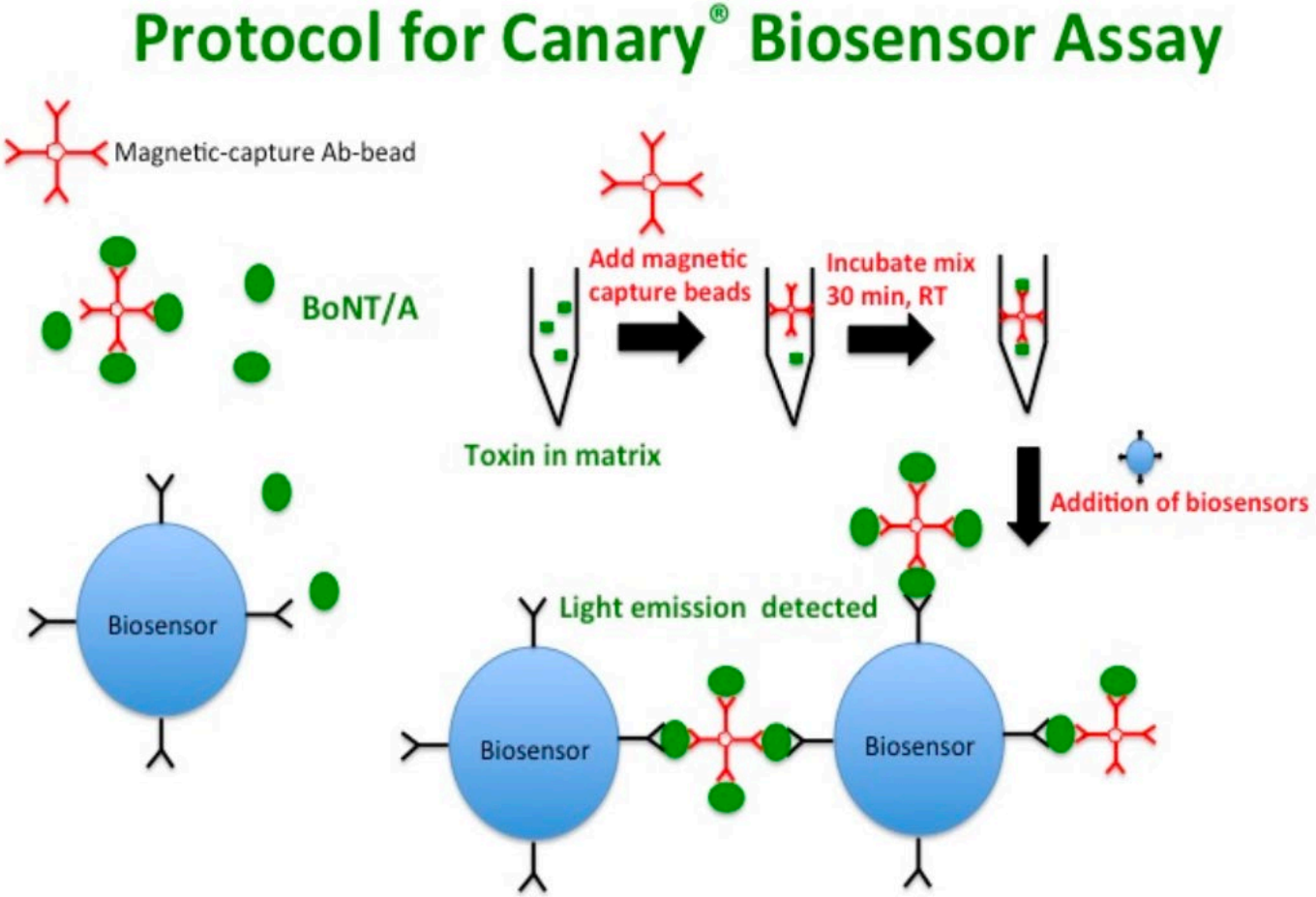
MDPI

Article

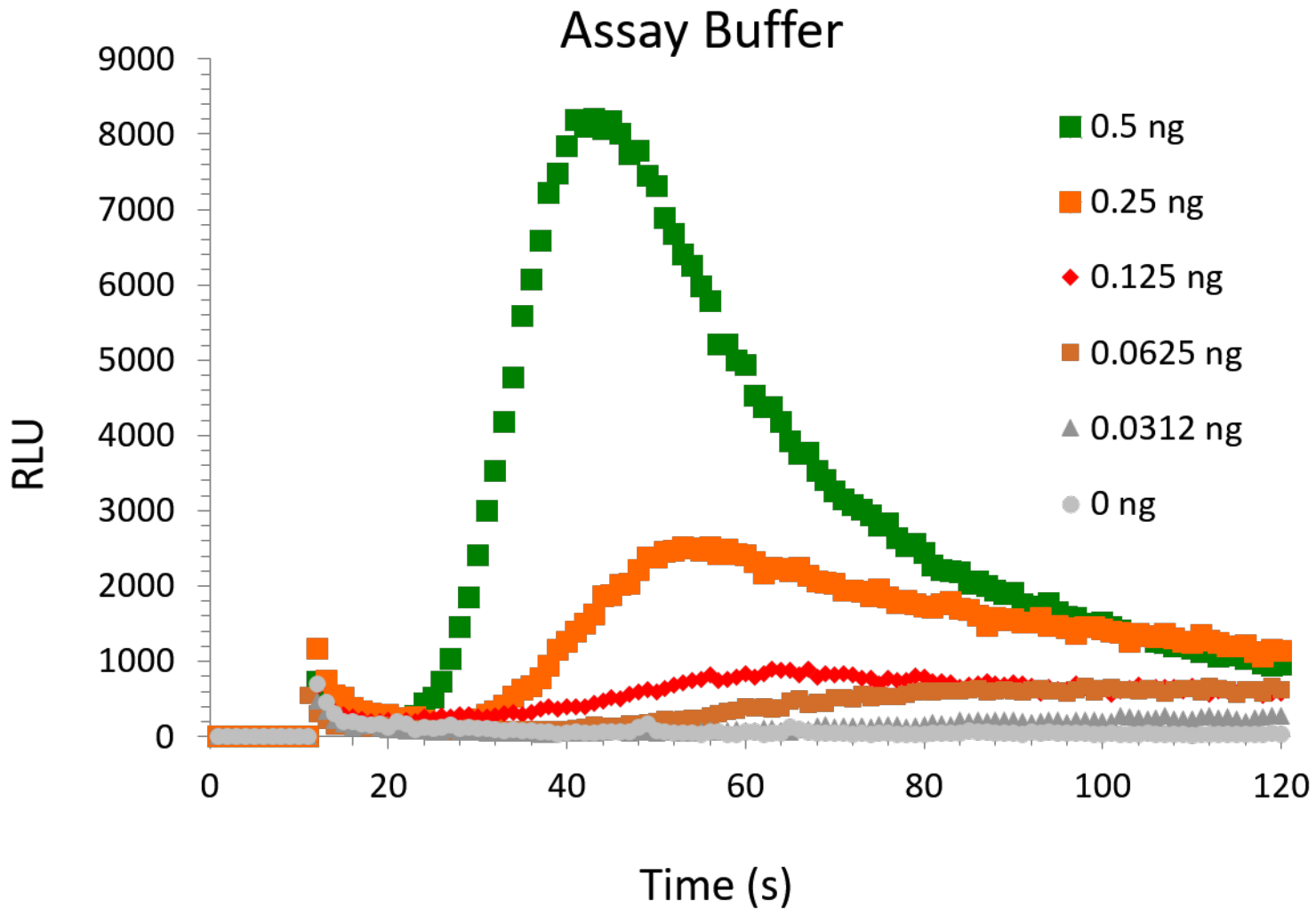
A Rapid, Sensitive, and Portable Biosensor Assay for the Detection of Botulinum Neurotoxin Serotype A in Complex Food Matrices

Christina C. Tam [1], Andrew R. Flannery [2] and Luisa W. Cheng [1,*]

- Rapid detection of BoNT/A ≈ 40 min
- Highly sensitive, small sample volume
- Minimal sample manipulation
- Antibody-conjugated magnetic beads for capture
- B-cell biosensor expressing antibodies
- Capture mAb-Antigen-B cell interaction induces luminescence output
- Platform for development of sensitive and rapid detection of toxins and pathogens



BoNT/A detection in Assay Buffer



BoNT/A (ng)	Read Out
0.5	Positive
0.25	Positive
0.125	Positive
0.0625	Positive
0.0312	Negative
0	Negative

- Detection limit of the assay is < 0.0625 ng of BoNT/A in assay buffer

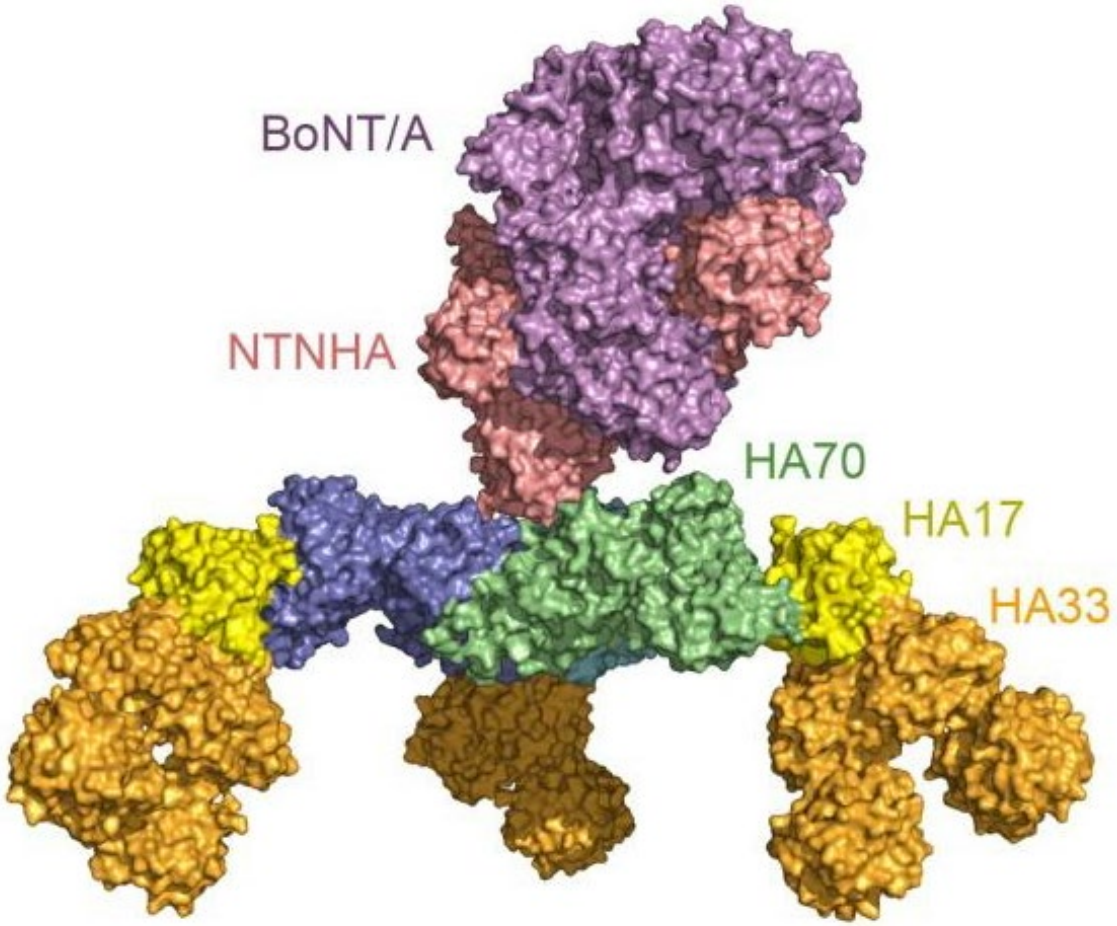
CANARY® Zephyr Detection of BoNT/A in the presence of food

Food Matrix	LOD (ng/mL)
Assay buffer	10.0 ± 2.5
Whole milk	7.4 ± 2.2
2% milk	7.9 ± 2.5
Non-fat milk	7.6 ± 2.3
Orange juice (Neutralized)	62.5 ± 21.2
Apple juice (Neutralized)	75.0 ± 21.6
Carrot juice (Neutralized)	32.5 ± 12.0

Food Matrix	LOD (ng/mL)
Liquid egg (Diluted)	171.9 ± 64.7
Ground beef	14.8 ± 2.6
Green bean baby food	16.6 ± 6.5
Smoked salmon	62.5 ± 0.00

CANARY® Zephyr detects BoNT/A in 10 complex matrices with varying limits of detection.

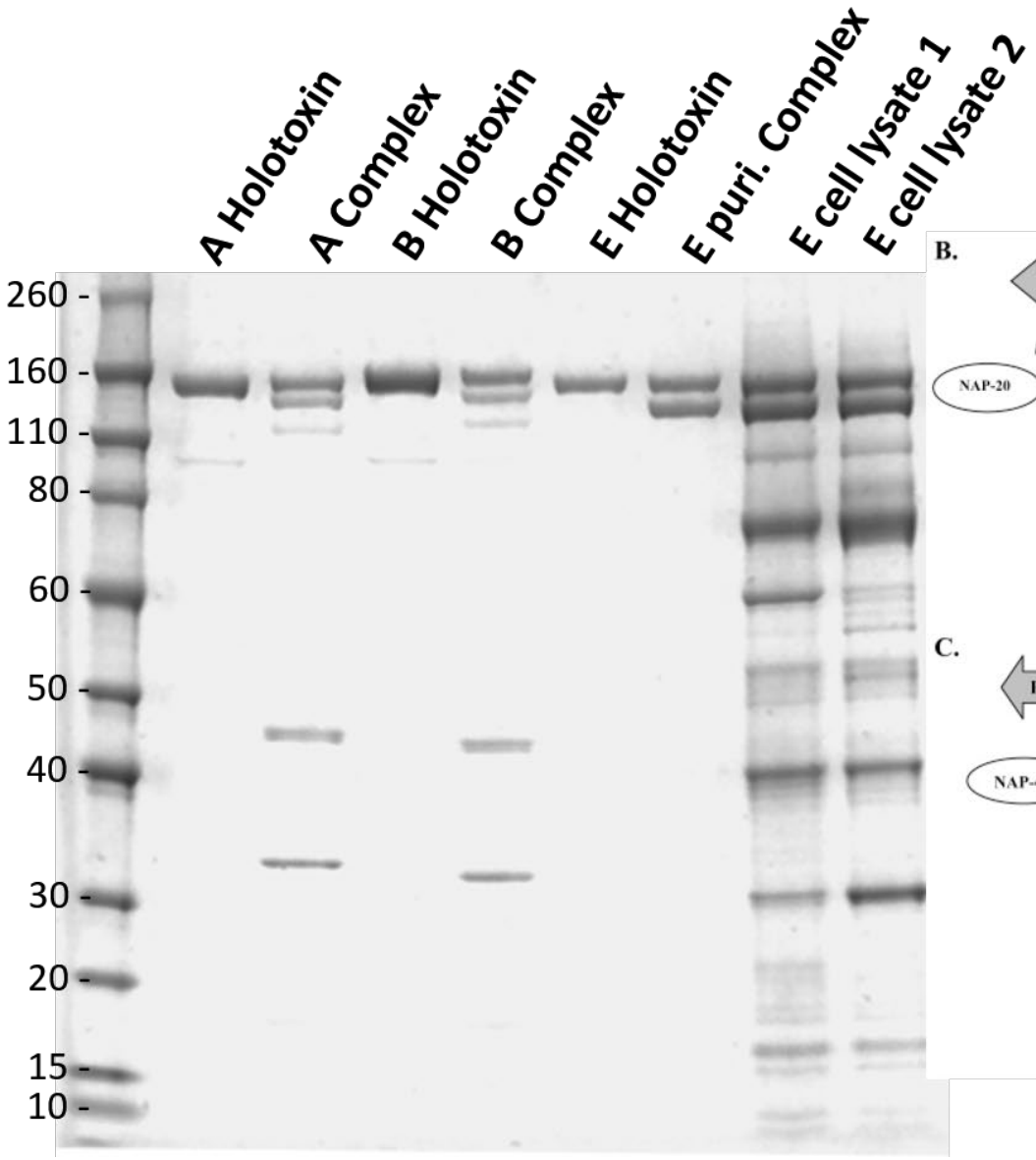
BoNT/A complex structure: effect of NAPs on oral toxicity



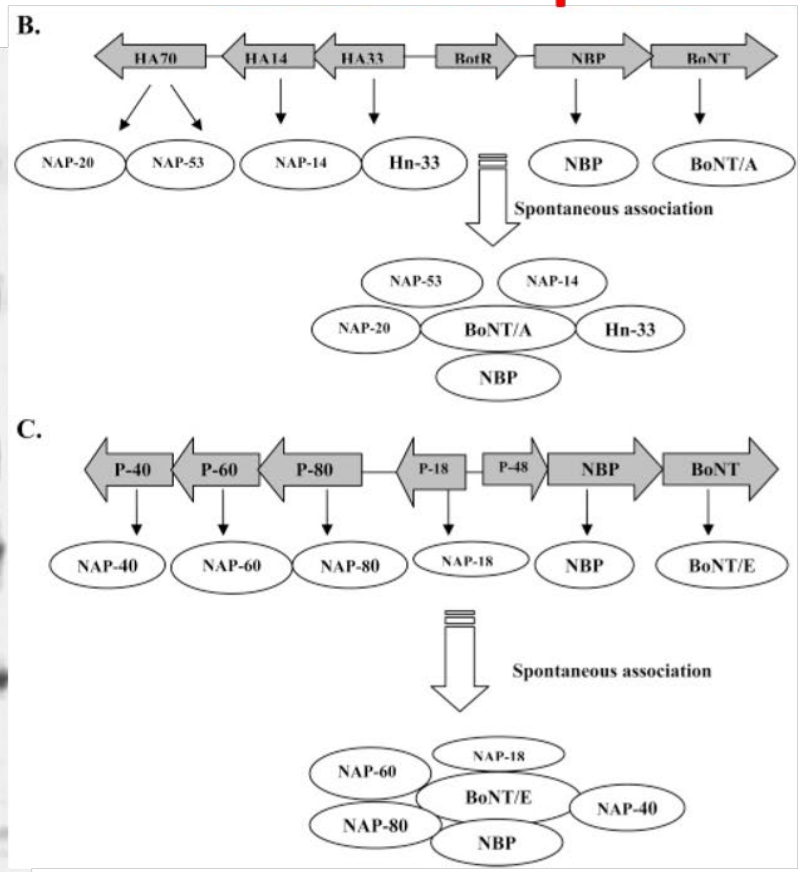
Toxin in complex is 20X more toxic than holotoxin in oral assays

Lee et. al, *Plos Pathogens* 2013

Protein components of BoNT complexes



NAPs: Neurotoxin associated proteins



Kukreja and Singh 2007, *Biochemistry* (6)

Oral bioavailability of BoNT complexes in phosphate gelatin

Oral: intragastric inoculation

Toxin*	Oral LD_{50}'s (µg/kg)
Purified A complex	27 ± 3
Crude A extract	30 ± 3
BoNT/B	0.24 ± 0.028
Purified E complex	535
Crude E cell lysate	24 ± 1

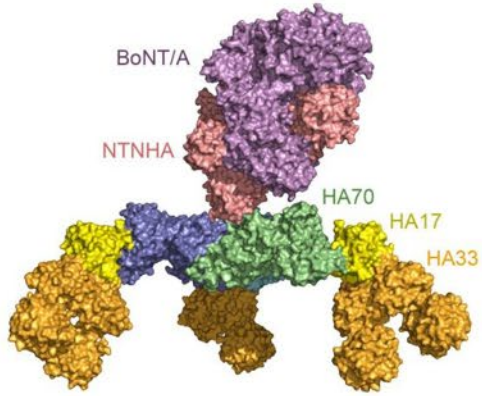
Cheng et al, unpublished results

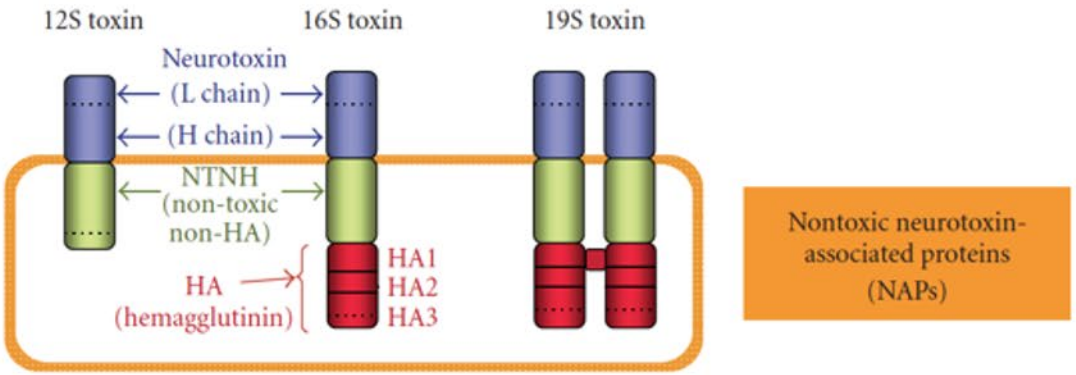
- Purified BoNT/E complex only consists of E holotoxin and NTNHA
- Presence of other neurotoxin associated proteins (NAP) in crude extract enhanced oral toxicity of BoNT/E
- Carbohydrate receptor mimics block intestinal receptor binding of NAP, reducing oral toxicity

Inhibiting oral intoxication of botulinum neurotoxin A complex by carbohydrate receptor mimics

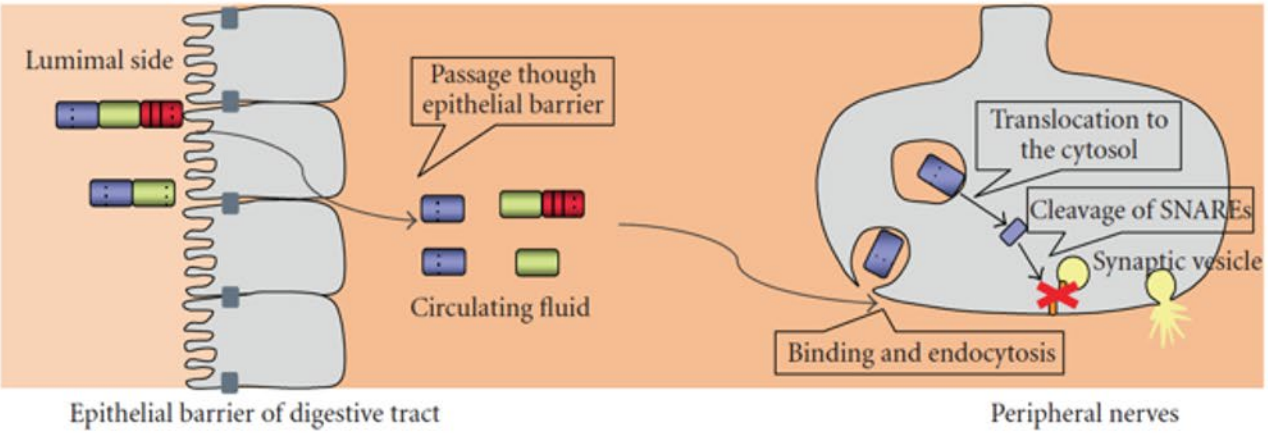
Kwangkook Lee[1,†], **Kwok-Ho Lam**[1,†], **Anna-Magdalena Kruel**[2], **Stefan Mahrhold**[2], **Kay Perry**[3], **Luisa W. Cheng**[4], **Andreas Rummel**[2,*], and **Rongsheng Jin**[1,*]

Role of NAPs in Botulinum Neurotoxin (BoNT) epithelial cell binding and internalization





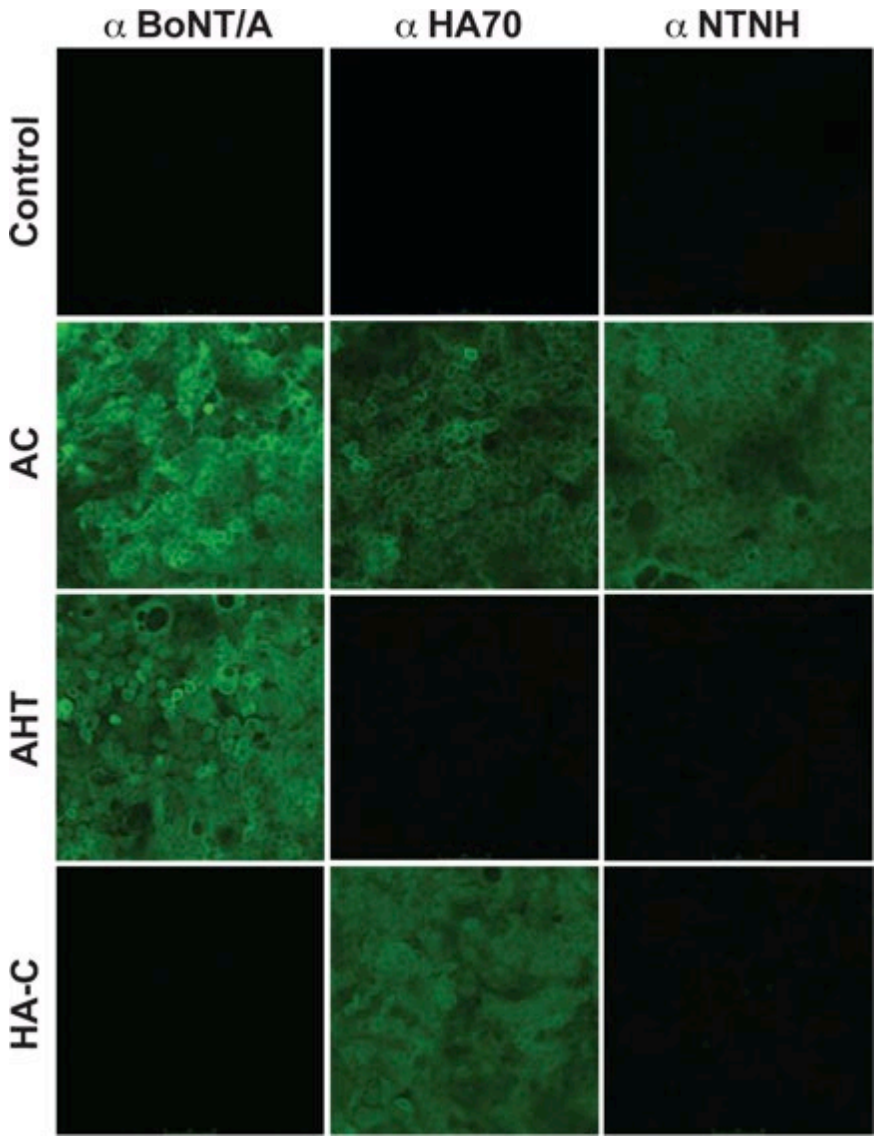
(a) BoNT complex



(b) Food-borne botulism

Fujinaga, 2010 *Journal of Biomedicine and Biotechnology*

Uptake of BoNT/A complex, holotoxin, and HA complex in Caco-2 cells



Translocation of botulinum neurotoxin serotype A and associated proteins across the intestinal epithelia

Tina I. Lam,[1] Larry H. Stanker,[1] Kwangkook Lee,[2] Rongsheng Jin[2] and Luisa W. Cheng[1]*

[1]*Foodborne Toxin Detection and Prevention Unit, Western Regional Research Center, U.S. Department of Agriculture -Agricultural Research Service, Albany, CA 94710, USA.*

[2]*Physiology & Biophysics, School of Medicine, University of California, Irvine, CA 92697, USA.*

BoNT/A toxin complex translocation whereby toxin complex entry is facilitated by NAPs in a receptor-mediated mechanism. Understanding the intestinal uptake of BoNT complexes will aid the development of new measures to prevent or treat oral intoxications.

4 h after toxins (labeled in green fluorescence) added

Lam et al., Cellular Microbiology, 2015

BoNT in mouse intestines

No Toxin

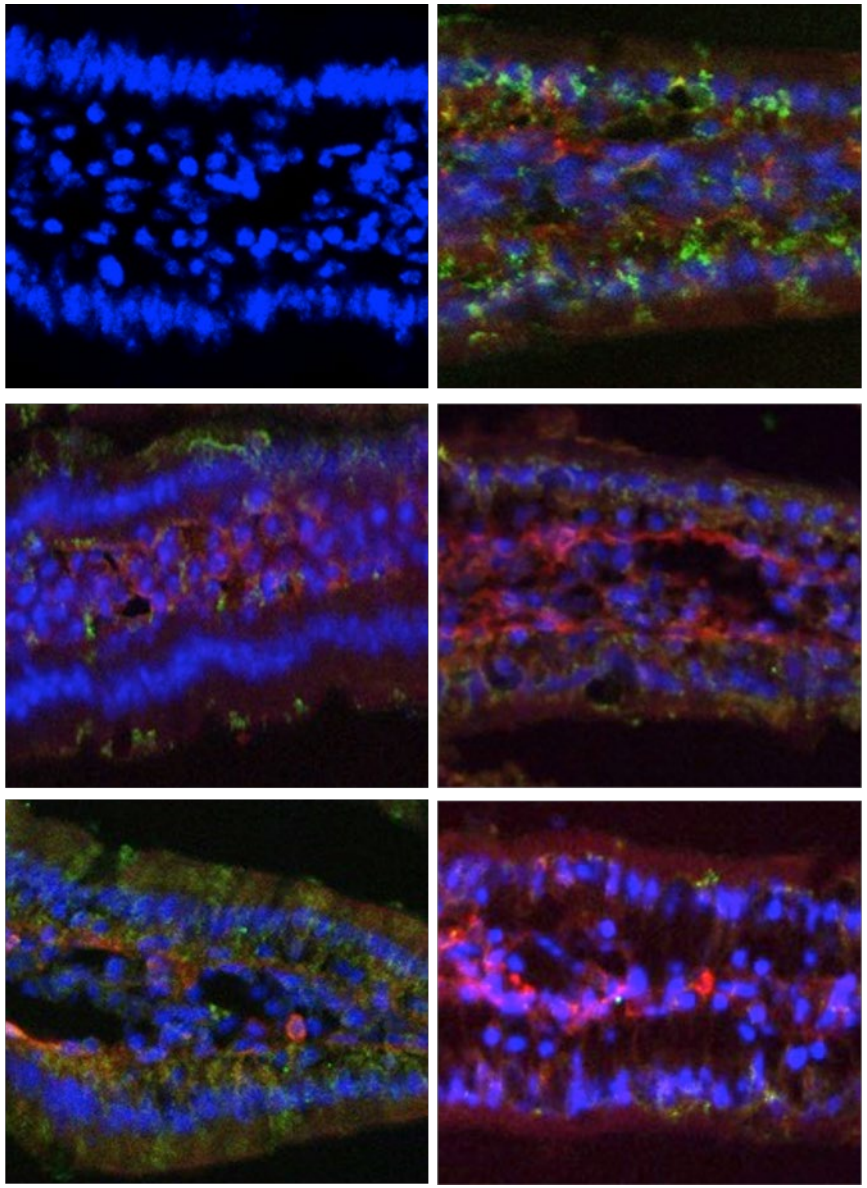
6 h

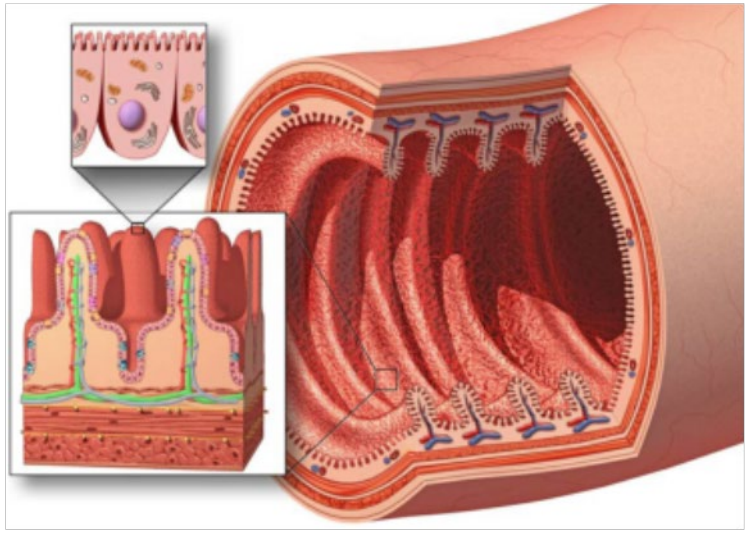
2 h

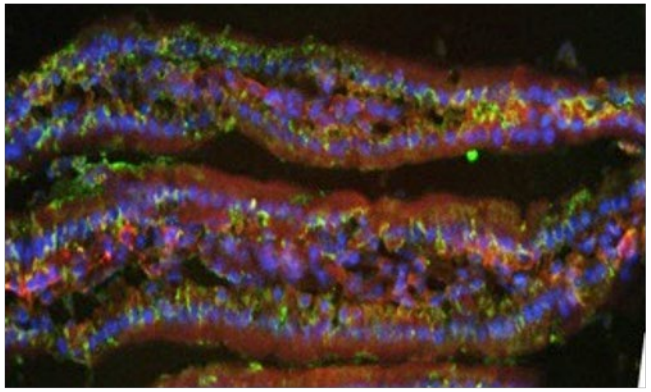
8 h

4 h

24 h



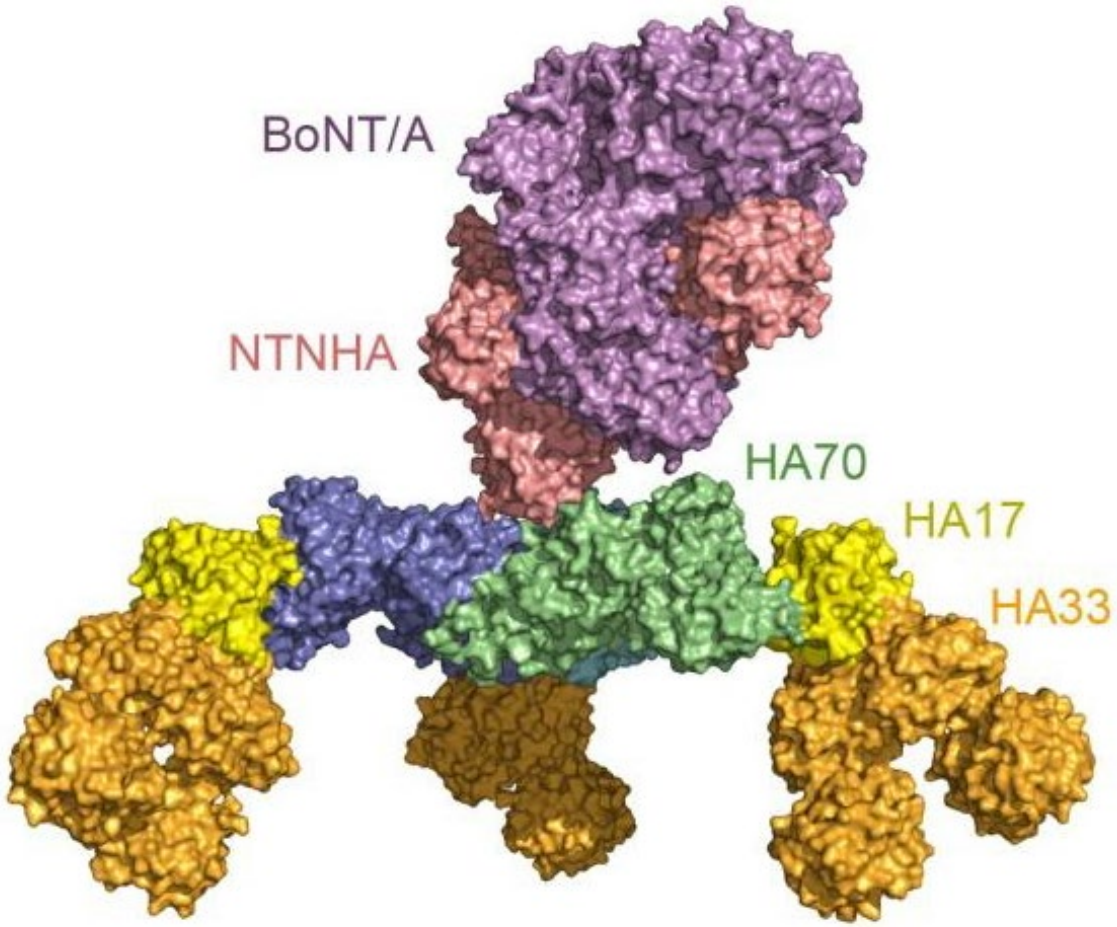




Green:=toxin **Red:=HA70**

Lam et al., Cellular Microbiology, 2015

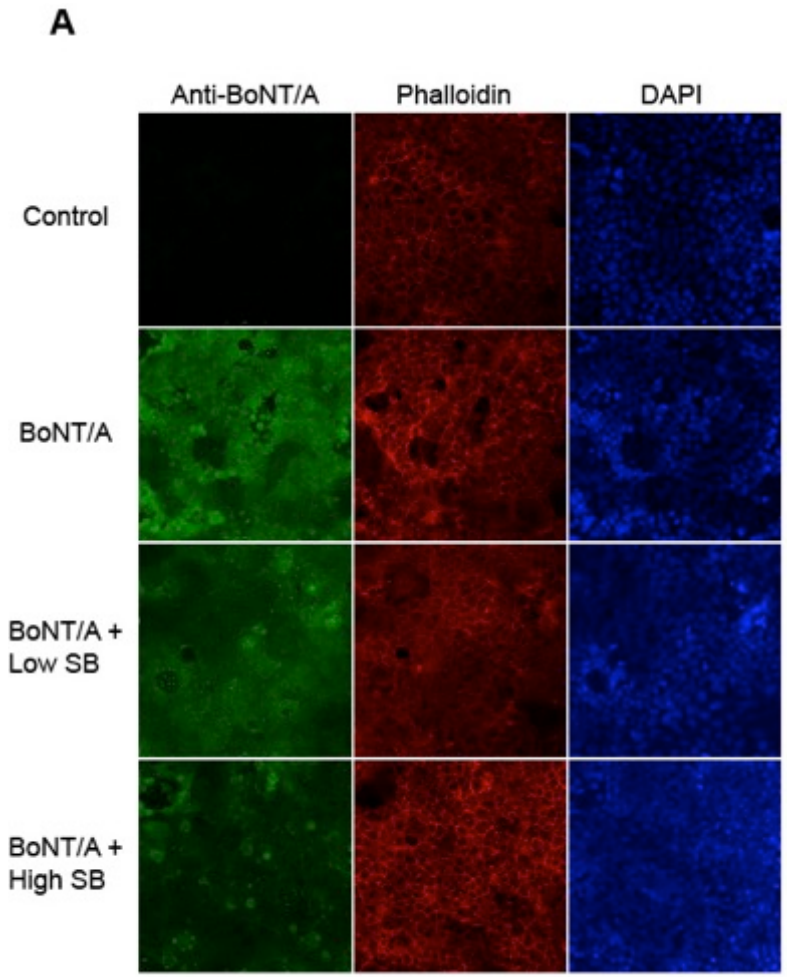
BoNT/A complex structure

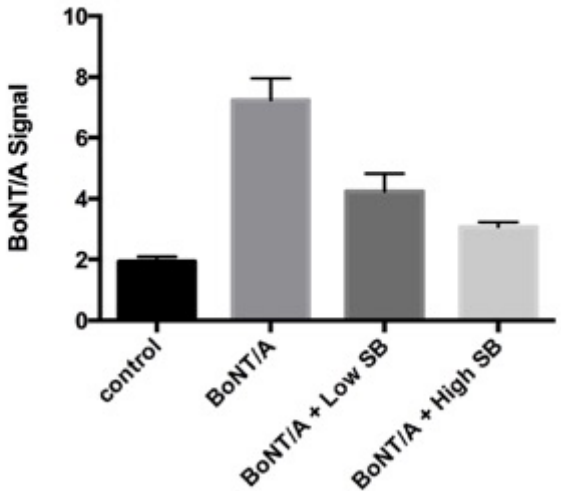


NAPs are not necessary for toxin absorption/intestinal binding but speeds up uptake by ~ 2 hr

Lee et. al, *Plos Pathogens* 2013

S. cerevisiae var. *boulardii* reduced BoNT/A binding to intestinal cells





toxins

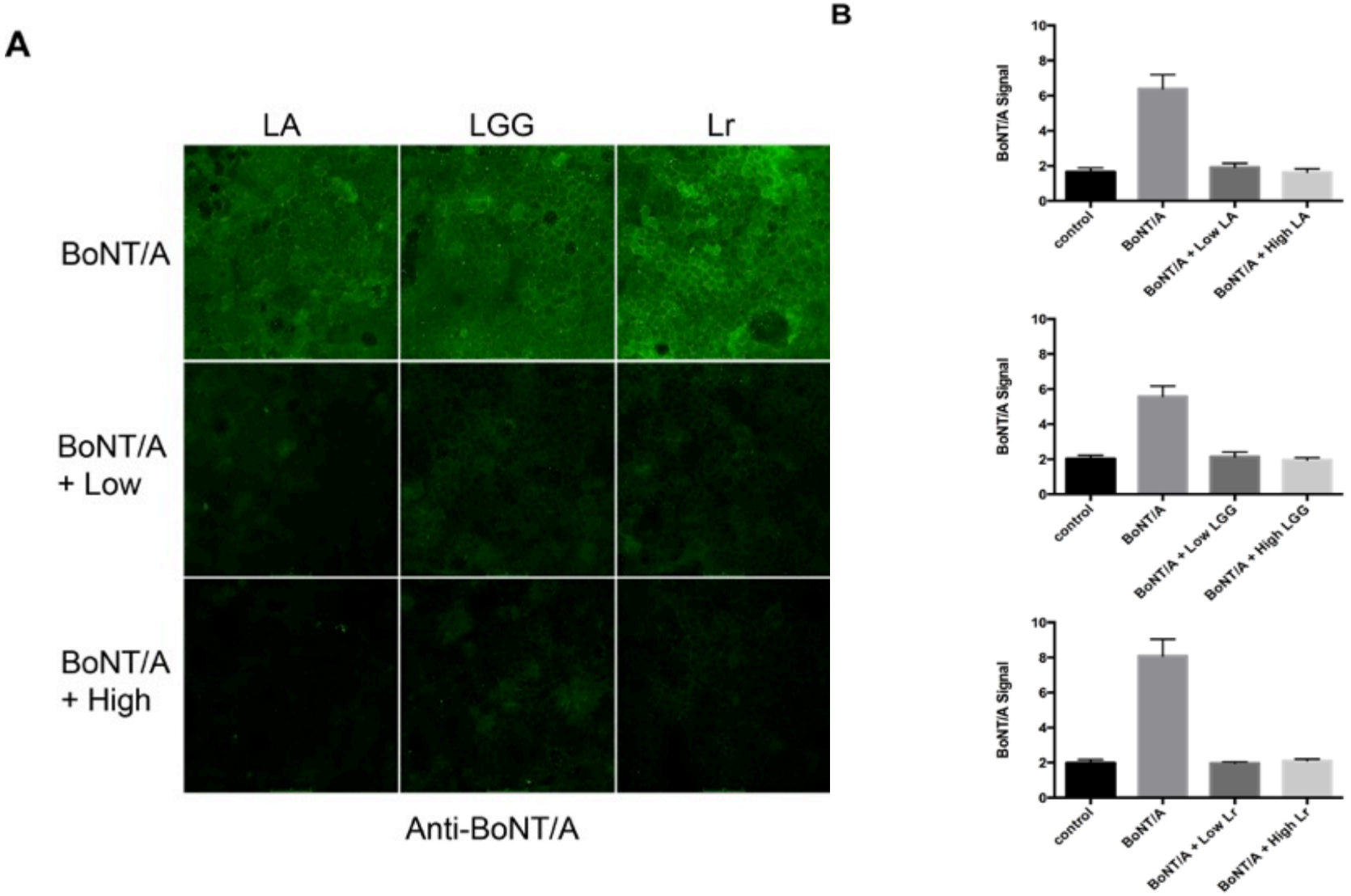
MDPI

Article

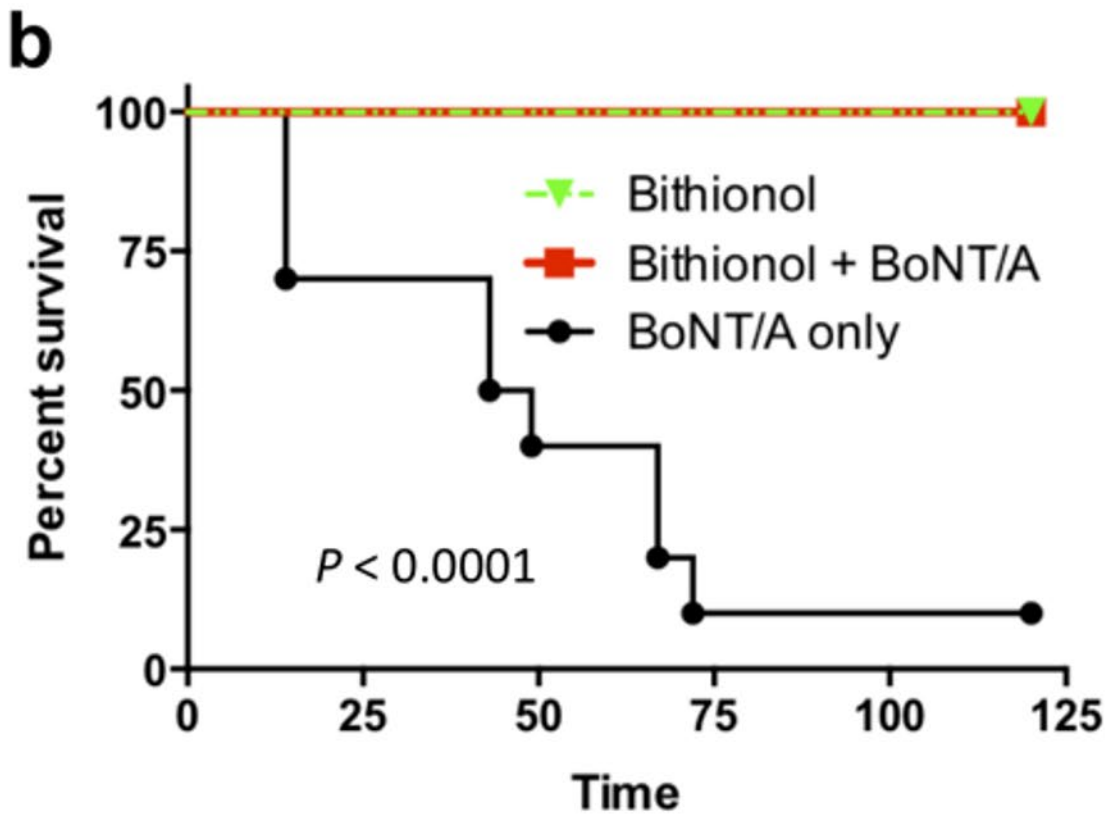
Probiotic Microorganisms Inhibit Epithelial Cell Internalization of Botulinum Neurotoxin Serotype A

Tina I. Lam †, Christina C. Tam †, Larry H. Stanker and Luisa W. Cheng *

Lactobacillus strains reduced BoNT/A binding to intestinal cells



BoNT/A oral toxicity is abrogated by Bithionol treatment

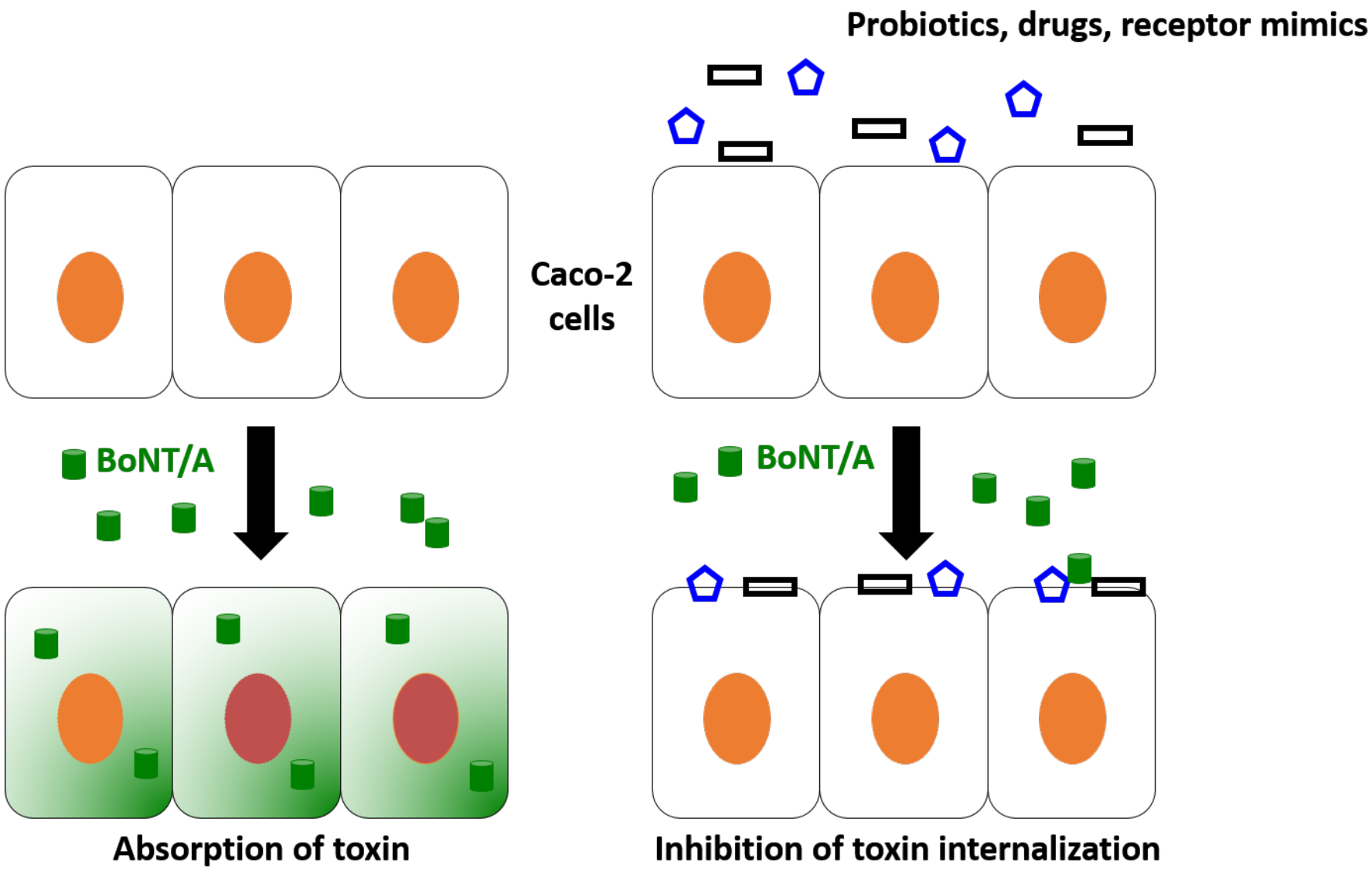


3 μg/mouse BoNT/A complex
6 mg/kg of bithionol

Bithionol blocks pathogenicity of bacterial toxins, ricin, and Zika virus

William Leonardi[1], Leeor Zilbermintz[1], Luisa W. Cheng[2], Josue Zozaya[1], Sharon H. Tran[1], Jeffrey H. Elliott[1], Kseniya Polukhina[1], Robert Manasherob[3], Amy Li[3], Xiaoli Chi[4], Dima Gharaibeh[4], Tara Kenny[4], Rouzbeh Zamani[4], Veronica Soloveva[4,5,6], Andrew D. Haddow[4], Farooq Nasar[4], Sina Bavari[4], Michael C. Bassik[3], Stanley N. Cohen[3], Anastasia Levitin[1] & Mikhail Martchenko[1]

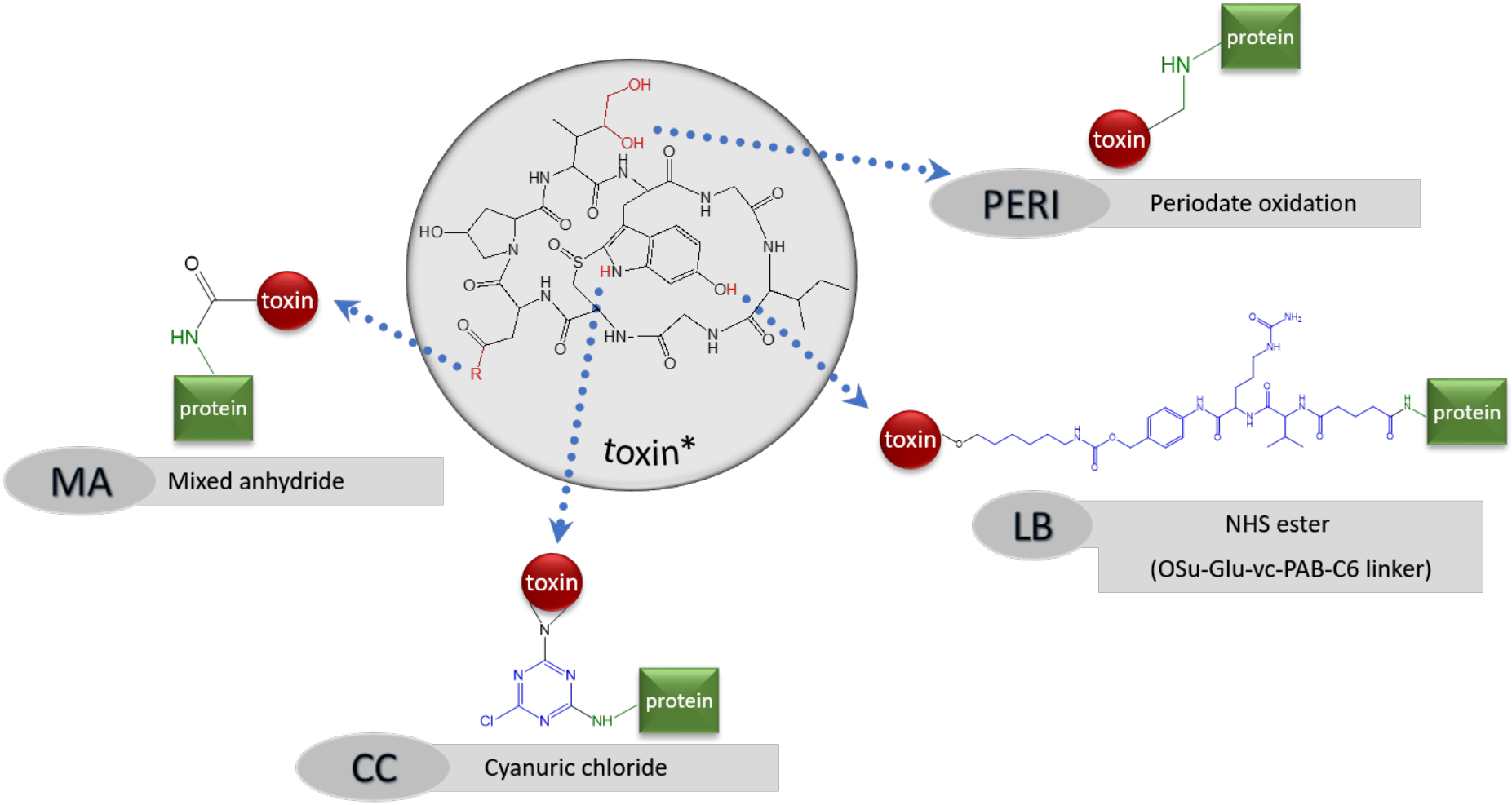
Summary


Probiotics, drugs, receptor mimics
Caco-2 cells
BoNT/A
BoNT/A
Absorption of toxin
Inhibition of toxin internalization

Amatoxins

- Develop monoclonal and polyclonal antibodies to amatoxins
- Immunoassays: ELISA, lateral flow assays

Antigen/Immunogen synthesis for amatoxin conjugates



* α-amanitin R=NH_2
β-amanitin R=OH

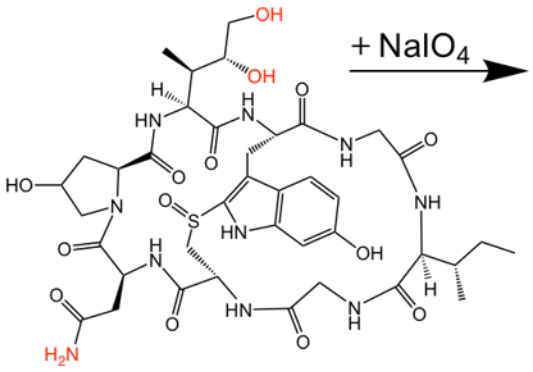
Red atoms indicate leaving groups
Blue atoms indicate linking arm

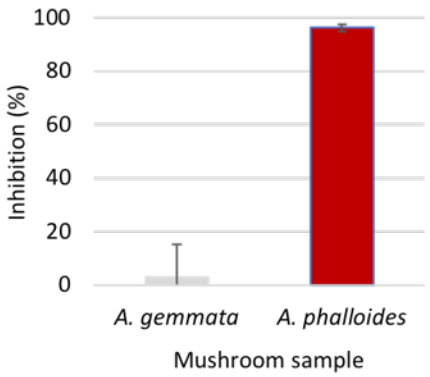
USDA Agricultural Research Service

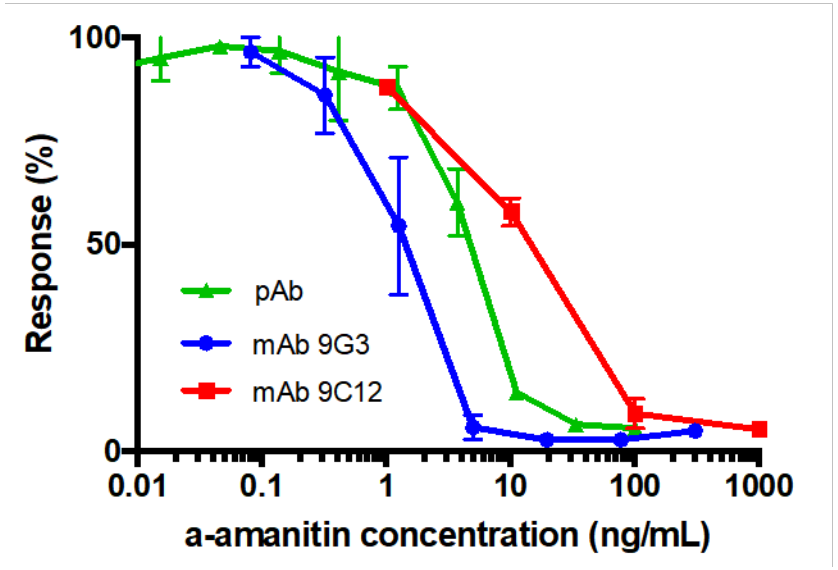
Bever et al, 2018, *Toxins*, 10(7)265

Antibodies to amatoxins

- Polyclonal antibody



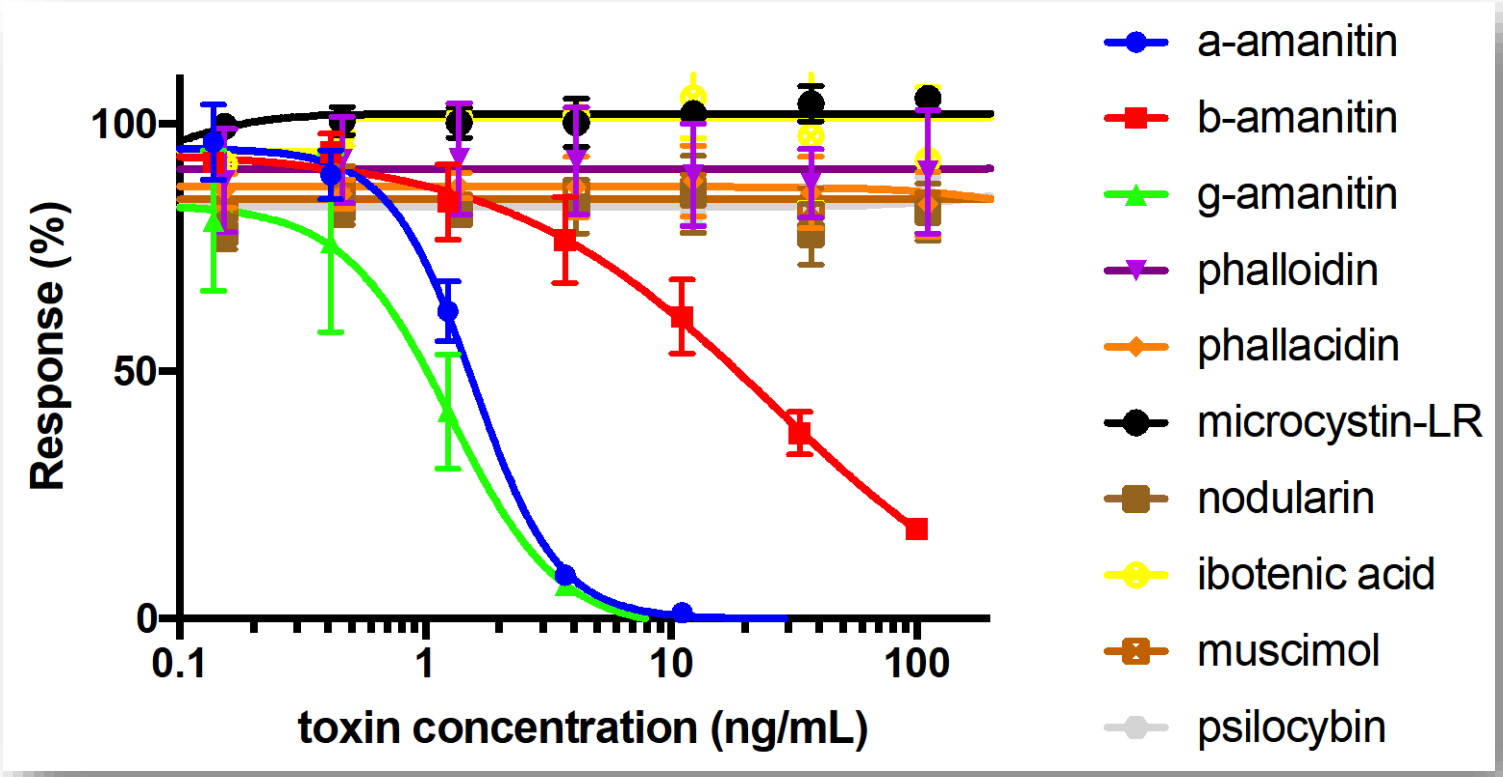




Antibody	K_d (pM)	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})
pAb rAb #58	35.0	$4x10^4$	$1.5x10^{-6}$
mAb 9G3	63.8	$1.2x10^5$	$7.8x10^{-6}$
mAb 9C12	823	$2x10^6$	$2x10^{-3}$

Bever and Stanker, unpublished results

ELISA for the detection of α, β and ɣ amatoxins



Assay is selective for amatoxins (α, and ɣ, less for β) but not other similar toxins

mAb 9G3, plate coated with BSA-ama-peri @ 1 ug/mL

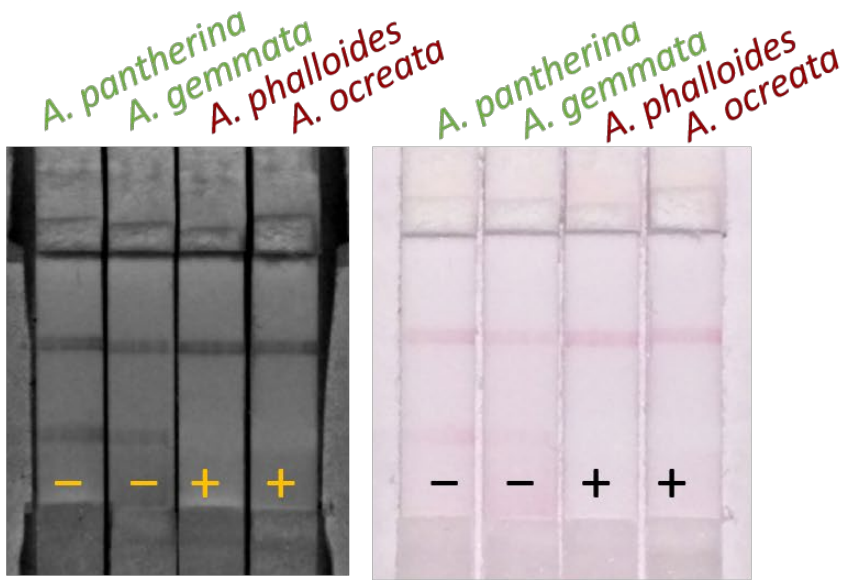
Lateral flow immunoassay: mushroom extracts

– +

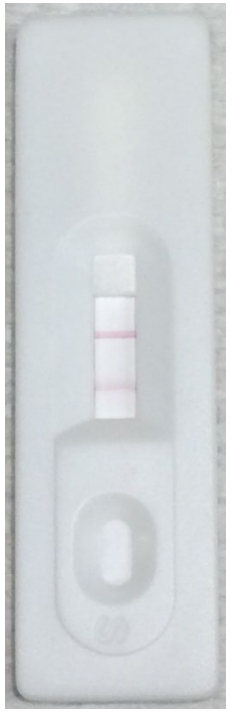
Amatoxins

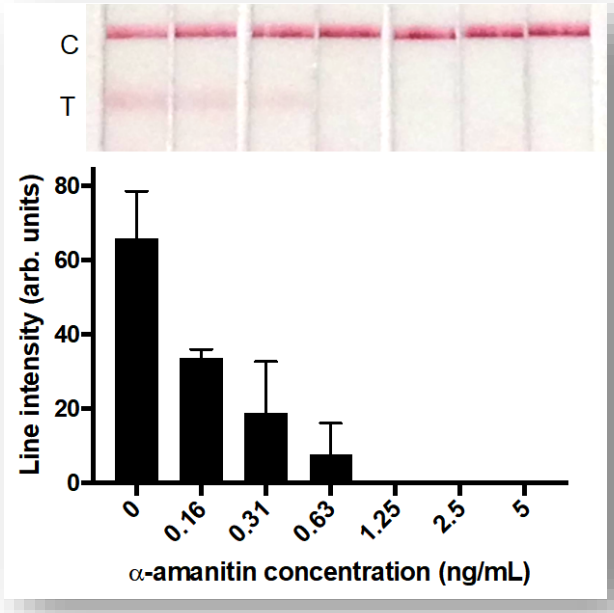
- Mushrooms from Dr. Bruns/Adams collection (UC Berkeley)
- ~20 mg of dried mushroom (cap) + 1mL PB
- Vortexed briefly
- 100 ul applied directly to LFA

Amatoxins

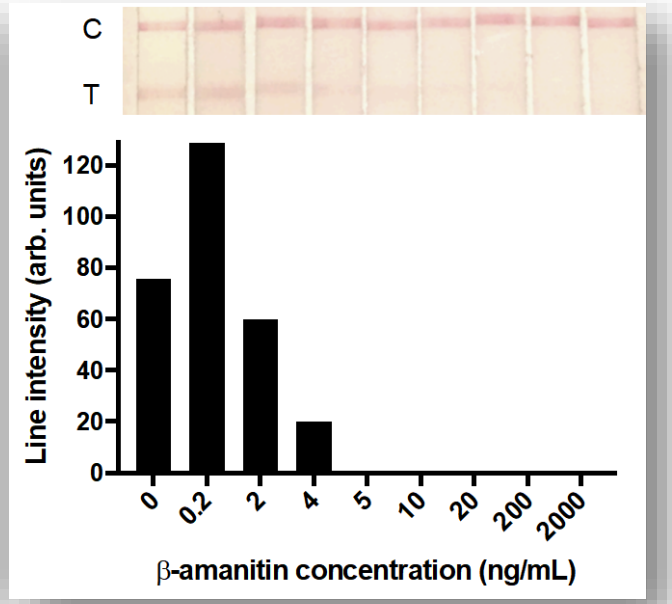


Lateral Flow Detection of Amanitins

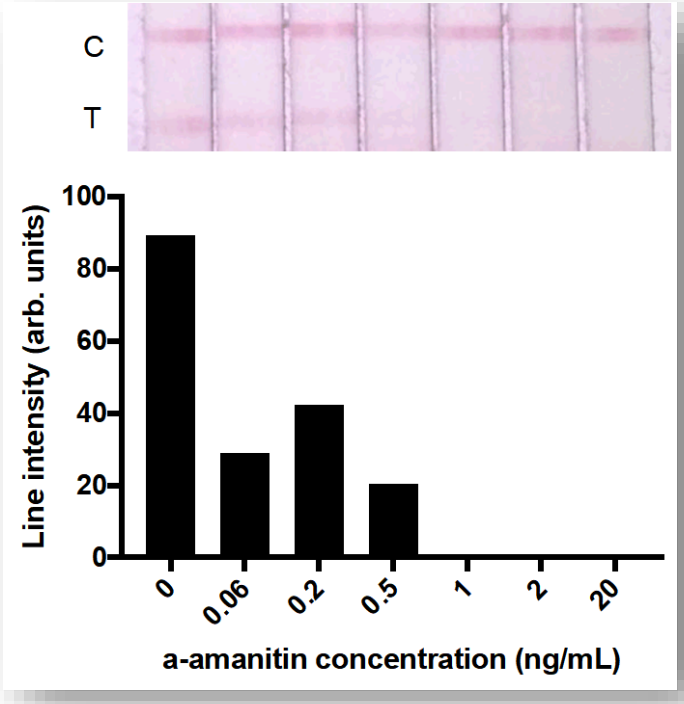




LOD: 1.25 ng/mL



LOD: 5 ng/mL



LOD: 1 ng/mL

Detection sensitivities for **α**-amanitin and **β**-amanitin

Direct urine analysis without the need for sample processing

Bever and Stanker, unpublished data

Abrin

- Develop monoclonal antibodies to abrin
- Compare in vitro and cell based activity assays with mouse bioassay
- Determine bioavailability of abrin after heat, pH and food processing
- Test neutralization of abrin +/- presence of antibodies

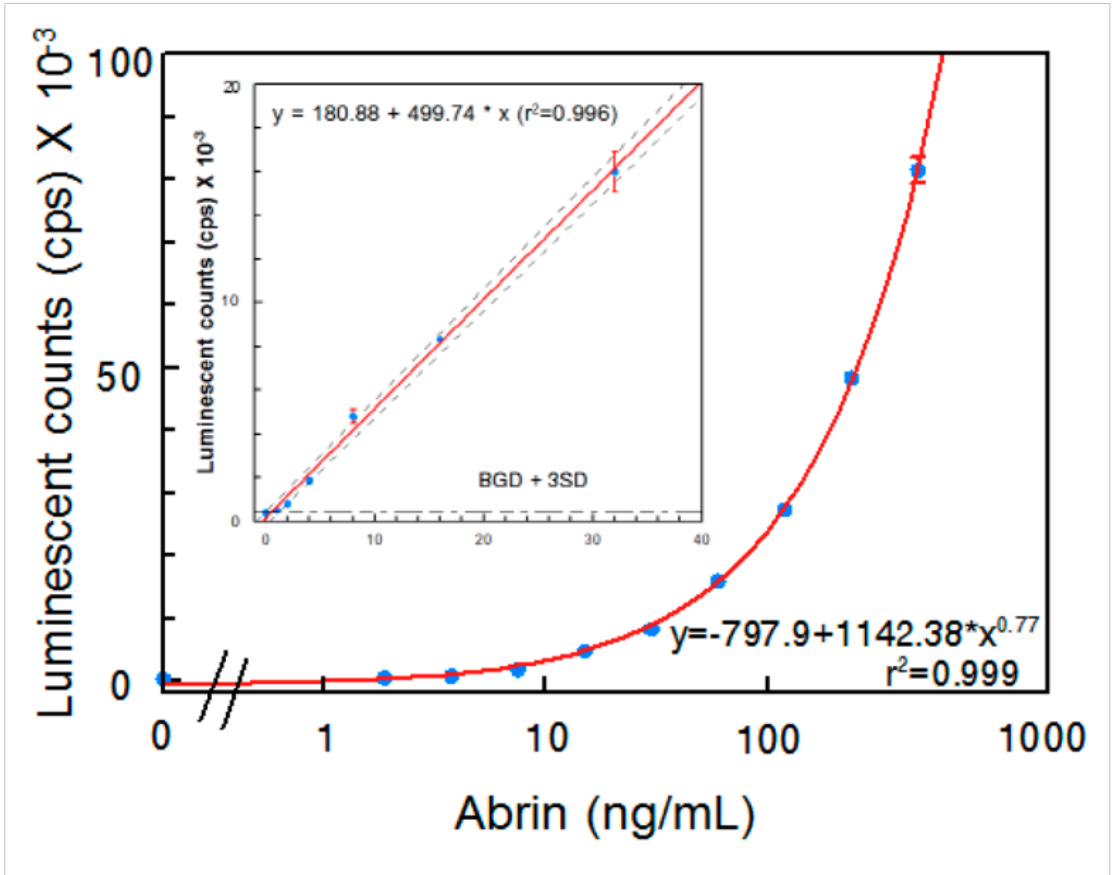
toxins

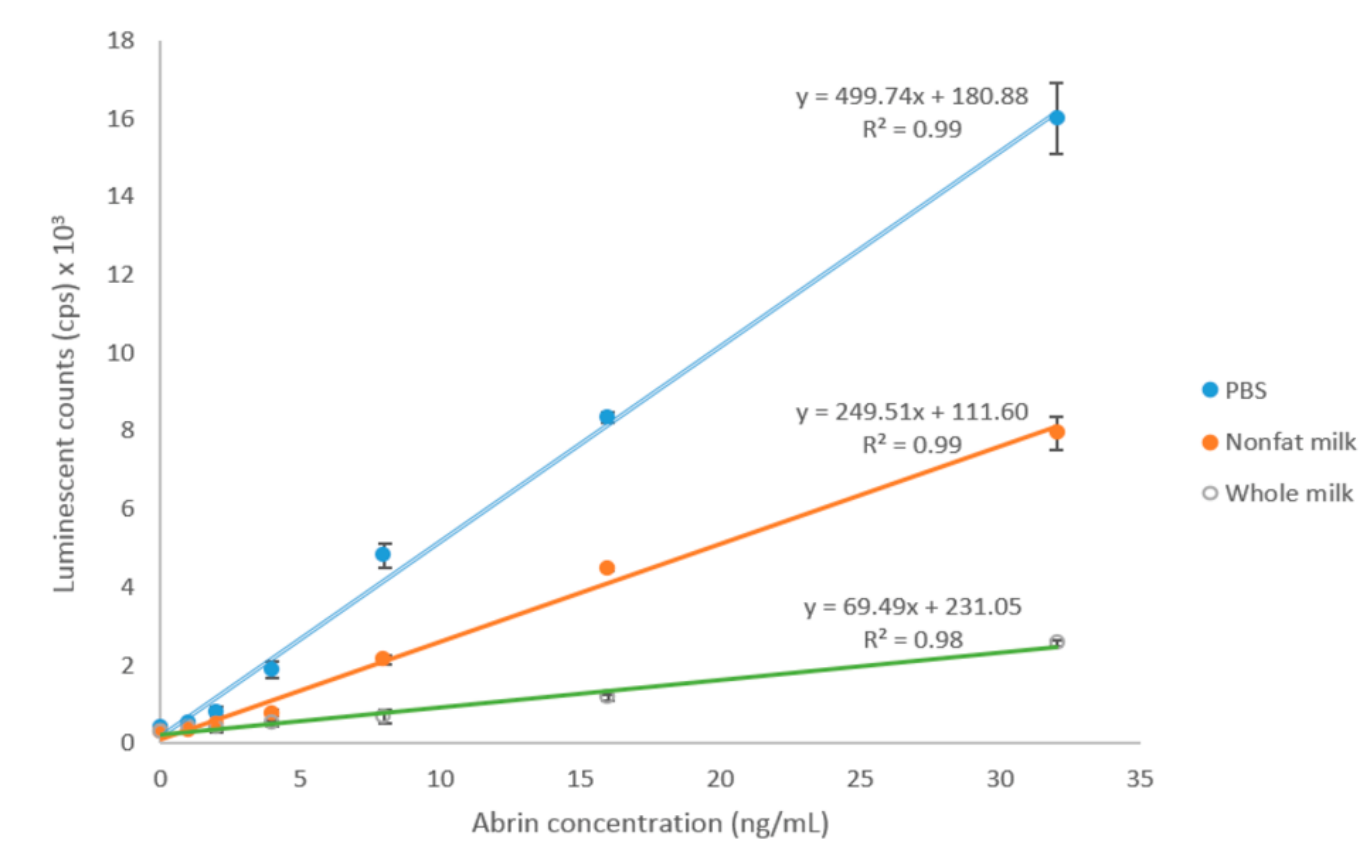
MDPI

Article

Detection of Abrin Holotoxin Using Novel Monoclonal Antibodies

Xiaohua He *, Stephanie Patfield, Luisa W. Cheng, Larry H. Stanker, Reuven Rasooly, Thomas A. McKeon, Yuzhu Zhang and David L. Brandon





Detection limit in buffer and milk matrices: 1 ng/ml

Name	Type	Western
LS02ABx	IgG1, kappa	Abrin holotoxin, A chain
LS03ABx	IgG1, kappa	Abrin holotoxin, A chain
LS04ABx	IgG2a, kappa	Abrin holotoxin, A chain
LS05ABx	IgG2b,kappa	Abrin holotoxin, A chain
LS06ABx	IgG1, kappa	Abrin holotoxin, A chain
LS07ABx	IgG1, kappa	Abrin holotoxin, A chain
LS08ABx	IgG2a, lambda	Abrin holotoxin, A chain
LS10ABx	IgG1, kappa	Abrin holotoxin, A chain
LS11ABx	IgG1, kappa	Abrin holotoxin, A chain
LS13ABx	IgG1, kappa	Abrin holotoxin, A chain

- **10 abrin mAbs were subtyped**

- **All mAbs were specific to**
- **abrin A-chain (catalytic domain)**

Detection limit in buffer and milk matrices: 1 ng/ml

toxins

MDPI

Article

A Monoclonal–Monoclonal Antibody Based Capture ELISA for Abrin

Christina C. Tam [1], Luisa W. Cheng [1], Xiaohua He [1], Paul Merrill [1], David Hodge [2] and Larry H. Stanker [1,*]

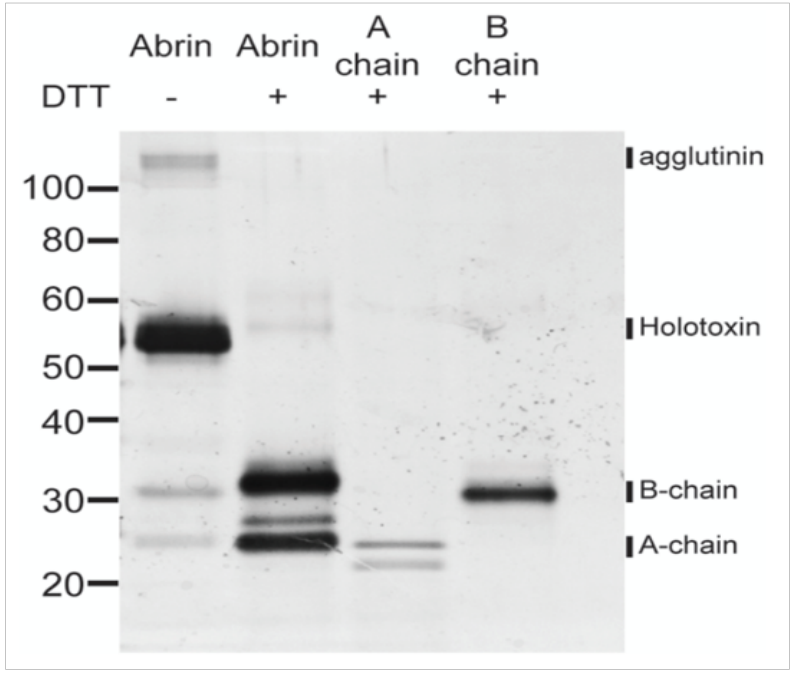


Table 3. Results of Abrin capture ELISA with near neighbor extracts.

Near Neighbours	Capture ELISA
Abrus laevigatus E. Mey.	-
Abrus precatorius L. +	+
Abrus schimperi subsp. *Africanus* (Vatke) Verdc.	-
Acalypha rhomboidea Raf.	-
Acalypha rhomboidea 11837	-
Adriana quadripartite (Labill.) Gaudich.	-
Adriana quadripartita 76851	-
Bryonia dioica Jacq.	-
Canavalia gladiate (Jacq.) DC.	-
Canavalia rosea (Sw.) DC	-
Canavalia madagascariensis J.D. Sauer	-
Cinnamomum camphora (L.) J.Presl.	-
Cucurbita moschata Duchesne	-
Dianthus caryophyllus Linnaeus	-
Fatsia japonica (Thunb.) Decne & Planch.	-
Fatsia japonica 39610	-
Galactia striata (Jacq.) Urban	-
Galactia wrightii A. Gray	-
Iris hollandica Bf.	-
Jubernardia globifera (Benth.) Troupin	-
Jubernardia globifera 76851	-
Luffa acutangula (L.) Roxb.	-
Luffa cylindrical (aegyptica) Bergquist, 1995	-
Lychnis chalcedonica (L.) E.H.L. Krause	-
Macaranga grandifolia (Blanco) Merr.	-
Macaranga grandifolia 3403	-
Mallotus nudiflorus (L.) Kulju & Welzen	-
Mallotus philippensis (Lam.) Müll. Arg.	-
Manihot escuelenta Crantz.	-
Manihot escuelenta 13562	-
Mercurialis annua L.	-
Mercurialis annua 279720	-
Momordica charantia L.	-
Phytolacca Americana L.	-
Phytolacca americana 39161	-
Phytolacca dioica L.	-
Plukenetia volubilis 72130	-
Plukenetia volubulis L.	-
Sambucus ebulus L.	-
Sambucus nigra L.	-
Saponaria officinalis L.	-
Saponaria officinalis 391915	-
Senna occidentalis (L.) Link	-
Trewia nudiflora 85123	-
Trichosanthes kirilowi Maxim.	-
Viscum album L.	-
Viscum album 22397	-

- = Zero activity at 3 μg/mL of sample in capture ELISA. + = Activity at 3 μg/mL of sample in capture ELISA.

Abrin Stability After Exposure to High Temperatures or pH

	63℃	74℃	80℃	85℃	99℃	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
Cell-free translation	+++	+++	+++	++	+	+++	+++	+++	+++	+++	+++	+++	+++
Cell culture cytotoxicity	+++	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++
Mouse bioassay	+++	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++

+++ Full toxicity
++ 50% toxicity
+ 25% toxicity
- No toxicity

toxins

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Article

Abrin Toxicity and Bioavailability after Temperature and pH Treatment

Christina C. Tam, Thomas D. Henderson II, Larry H. Stanker, Xiaohua He and Luisa W. Cheng *

Influence of Milk Matrices on the Thermal Inactivation of Abrin

	63°C 30 min	72°C 15 sec	134°C 2 sec	134°C 30 sec	134°C 60 sec	63°C 3min	74°C 3 min	80°C 3 min	85°C 3 min	99°C 3 min
Cell-free translation	+++	+++	+++	+++	++	+++	+++	+++	++	+
Cell culture cytotoxicity	+++	+++	+++	+++	+	+++	+++	+	+	+
Mouse bioassay	+++	+++	+++	+++	-	+++	+++	-	-	-

+++ Full toxicity
++ 50% toxicity
+ 25% toxicity
- No toxicity

toxins

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Article

Influence of Food Matrices on the Stability and Bioavailability of Abrin

Christina C. Tam, Thomas D. Henderson II, Larry H. Stanker and Luisa W. Cheng *

Stability and bioavailability of abrin in milk matrices

Temperature	Time	Inactivation Type
63 ℃	30 min	Vat Pasteurization
72 ℃	15 sec	High Temperature short time (HTST)
138 ℃	2 sec	Ultra Pasteurization (UP)
134 ℃	2 sec, 30 sec, **60 sec**	**Mimic UP**
63 ℃	3 min	Tam et al. 2017
74 ℃	3 min	Tam et al. 2017
80 ℃	**3 min**	**Tam et al. 2017**
85 ℃	**3 min**	**Tam et al. 2017**
99 ℃	**3 min**	**Tam et al. 2017**

- **Tested using CFT, cell cytotoxicity, mouse bioassay**
- **Complete inactivation (RED) requires temperatures ≥ 80℃ for 3 min or 134 ℃ for 60 sec**
- **No difference between non-fat and whole milk**
- **Abrin inactivation requires higher temperatures between buffer (Tam et al. 2017) and milk matrices**

International Dairy Foods Association suggested minimal processing times

Stability and bioavailability of abrin in liquid egg

Temperature	Time	Inactivation Type
60 ℃	2.4 min	Scrambled egg
60 ℃	3.5 min	Plain whole egg
63 ℃	3 min	Tam et al. 2017
74 ℃*	**3 min**	**Tam et al. 2017**
80 ℃**	**3 min**	**Tam et al. 2017**
85 ℃**	**3 min**	**Tam et al. 2017**
99 ℃**	**3 min**	**Tam et al. 2017**

- **Tested using CFT, cell cytotoxicity, mouse bioassay**
- **Complete inactivation requires temperatures $\geq$ 74℃ for 3 min**
- **Increasing temperatures 'cook' the liquid egg to generate 'solids' (*,**)**
- **Protection seen in vivo may due to testing of cleared supernatant and the toxin could be still 'active' in the solid form**

International Egg Pasteurization Manual suggested minimal processing times

Potential neutralization antibodies for abrin

Neutralization of abrin cytotoxicity with monoclonal antibodies-Vero cells

Treatment	Rel Tox (%)
DMEM	0
Abrin	100
LS07ABx	4 +3
LS13ABx	4 ±1
LS02ABx	4 ±3
LS02+07+13	4 ±3
LS04ABx+Abrin	94 ±1
LS07ABx+Abrin	69 ±2
LS08ABx+Abrin	88 ±1
LS10ABx+Abrin	94 ±1
LS13ABx+Abrin	54 ±1
LS11ABx+Abrin	77 ±1
LS03ABx+Abrin	96 ±0
LS02Ax+Abrin	66 ±2
LS02+07+13+Abrin	41 ±2

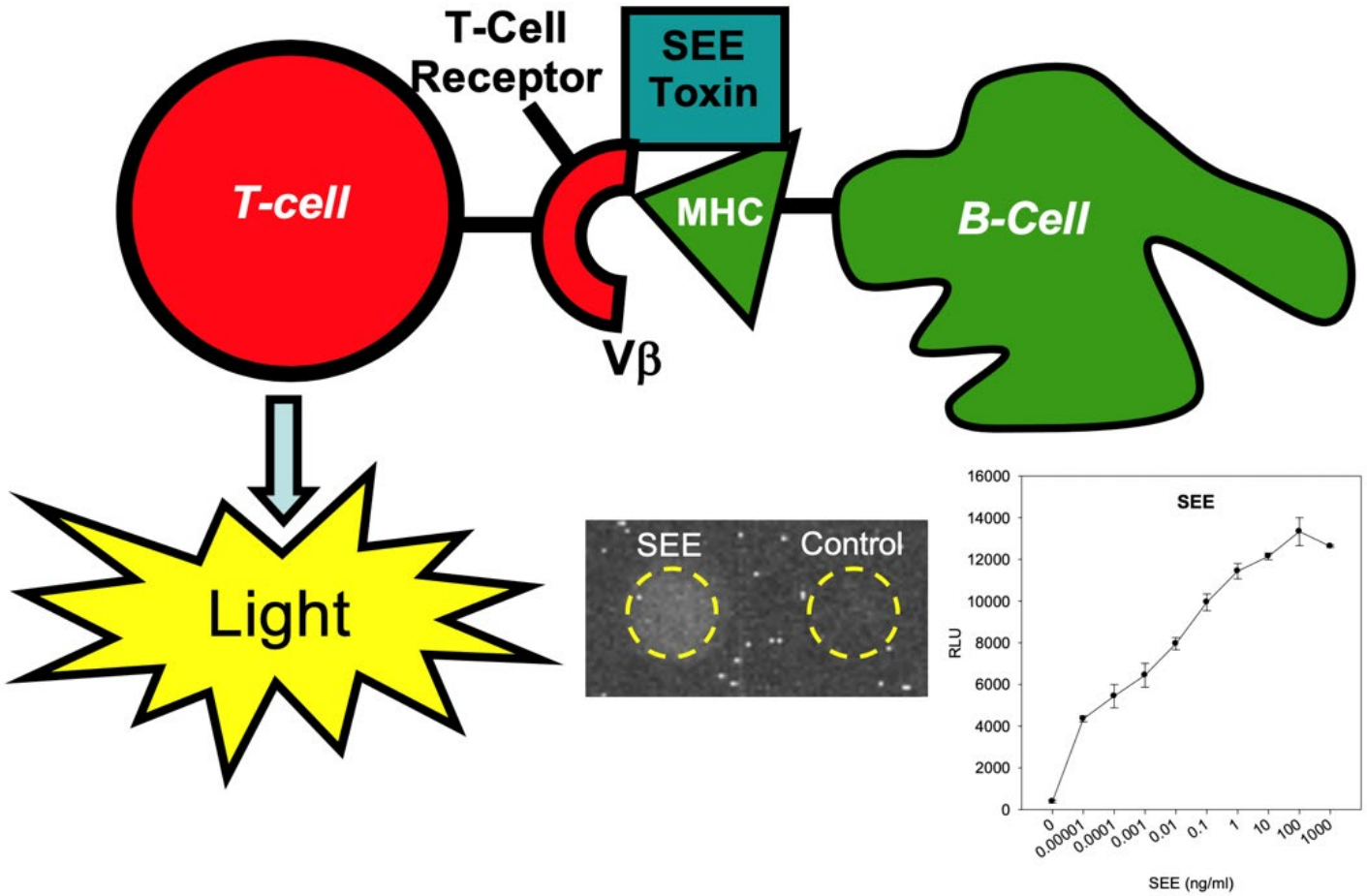
- **LS013ABx is most effective in inhibiting abrin cytotoxicity in a Vero cell assay**
- **A mix of LS02ABx/LS07ABx/LS13ABx mAbs enhances the protective effects seen with only LS13ABx**

Staph enterotoxins (SE)

- Develop novel T- cell based detection assay for SEE, SED and SEA
- Determine stability of SEs after heat processing

SE detection with T cell based assay

Figure 1. B-cells recognize SEE, present it to T-cells which then produce light. The light intensity corresponds to toxin concentration. The plate image shows T-cell glowing in the well.



Rapid Cell-Based Assay for Detection and Quantification of Active Staphylococcal Enterotoxin Type D

Reuven Rasooly, Paula M. Do, and Bradley J. Hernlem

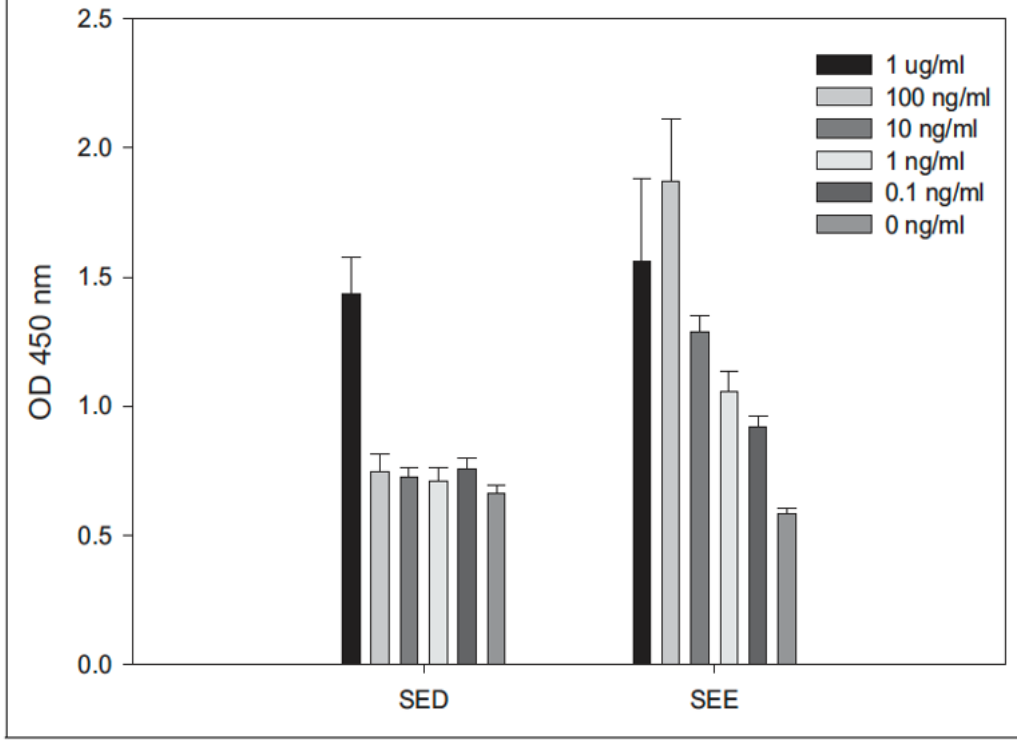


Figure 1–Detection of SED and SEE by splenocyte proliferation bioassay. Murine splenocytes were incubated with a range of concentrations of SED or SEE. After 5 d, BrdU incorporated in DNA of proliferating cells was measured as absorbance at 450 nm. Error bars represent standard errors.

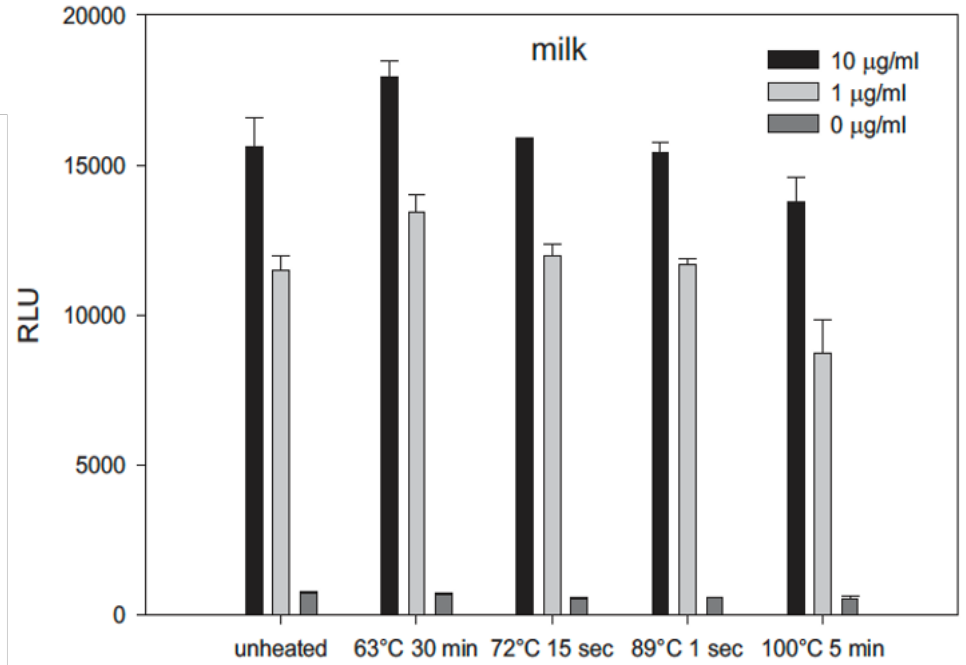


Figure 4–Pasteurization did not reduce SED toxin activity. Milk and PBS spiked with SED at concentrations of 10 or 1 μg/mL were pasteurized or treated at 100 °C for 5 min. Thermal treated spiked PBS or milk (15 μL) was added to Jurkat reporter cells. After incubation for 5 h, the luciferase enzyme activity was determined. Photon emission was detected by luminometer. Error bars represent standard errors.

LOD: 100 ng/ml SED

toxins

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Article

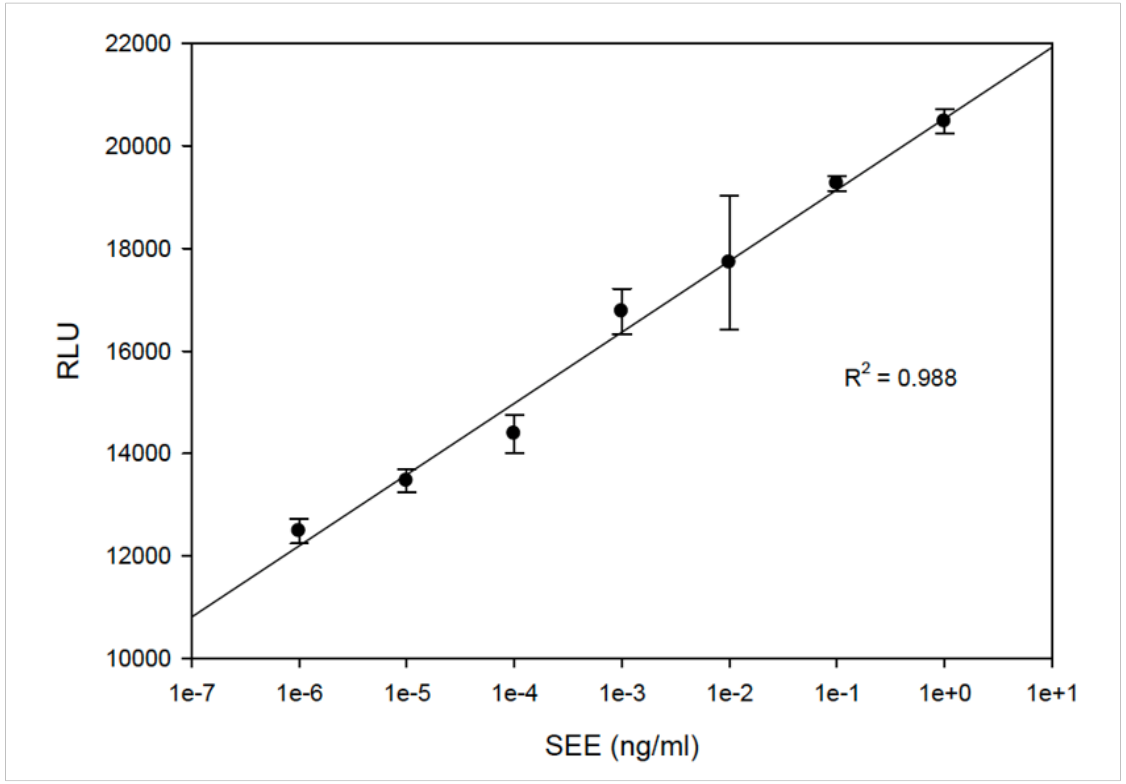
Sensitive, Rapid, Quantitative and *in Vitro* Method for the Detection of Biologically Active Staphylococcal Enterotoxin Type E

Reuven Rasooly *, Paula Do and Bradley Hernlem

J Food Prot. 2017 Oct 9:1857-1862. doi: 10.4315/0362-028X.JFP-17-196. [Epub ahead of print]

Interleukin 2 Secretion by T Cells for Detection of Biologically Active Staphylococcal Enterotoxin Type E.

Rasooly R[1], Do P[1], Hernlem BJ[1].



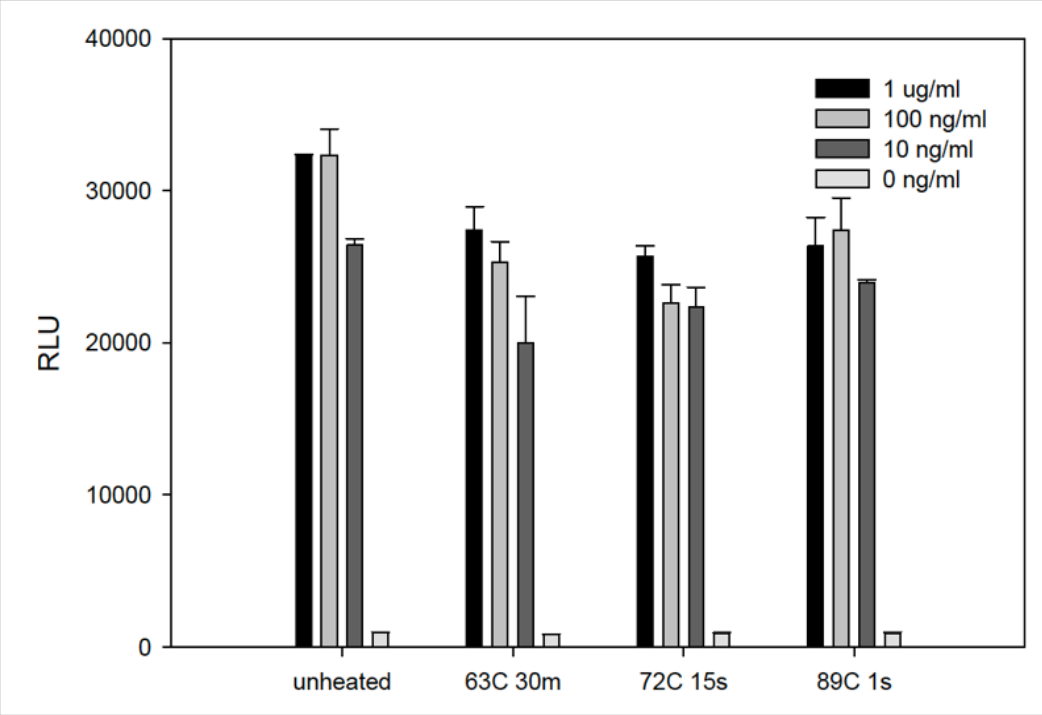


Figure 2. Bioluminescence assay for measuring biologically active SEE. Jurkat reporter cells were plated with Raji cells in a 96-well plate. The mixed cultures were co-incubated with various concentrations of SEE for 5 h. The photon emission was detected by luminometer. Error bars represent standard errors.

LOD: 1 fg/ml, 6 log linear range

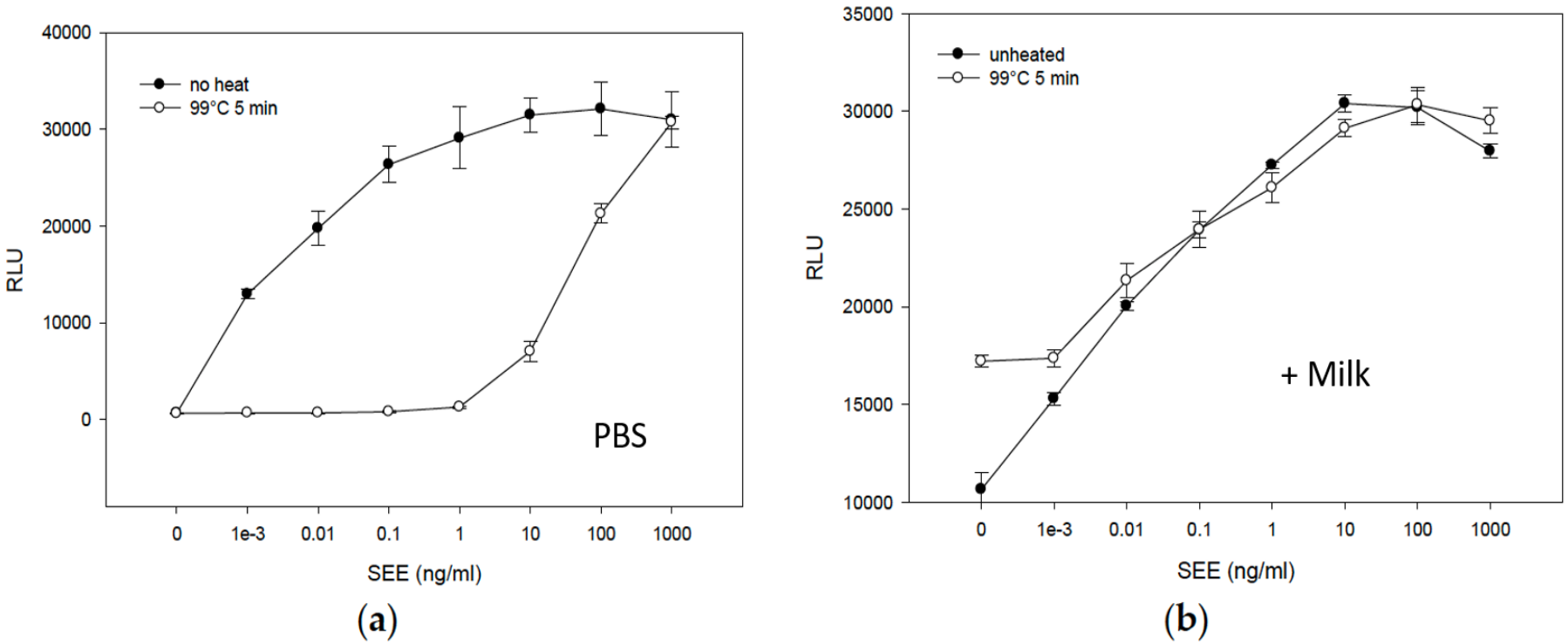


Figure 4. Thermal treatment at 99 °C for 5 min reduces the biological activity of SEE in PBS. However, milk has a protective effect on SEE retained from its initial activity. PBS (**a**) and milk (**b**) spiked with increasing concentrations of SEE were treated at 99 °C for 5 min. Thermal treated spiked PBS or milk (15 µL) was added to the Jurkat reporter cells. After incubation for 5 h, the luciferase enzyme activity was determined. Photon emission was detected by luminometer. Error bars represent standard errors.

toxins

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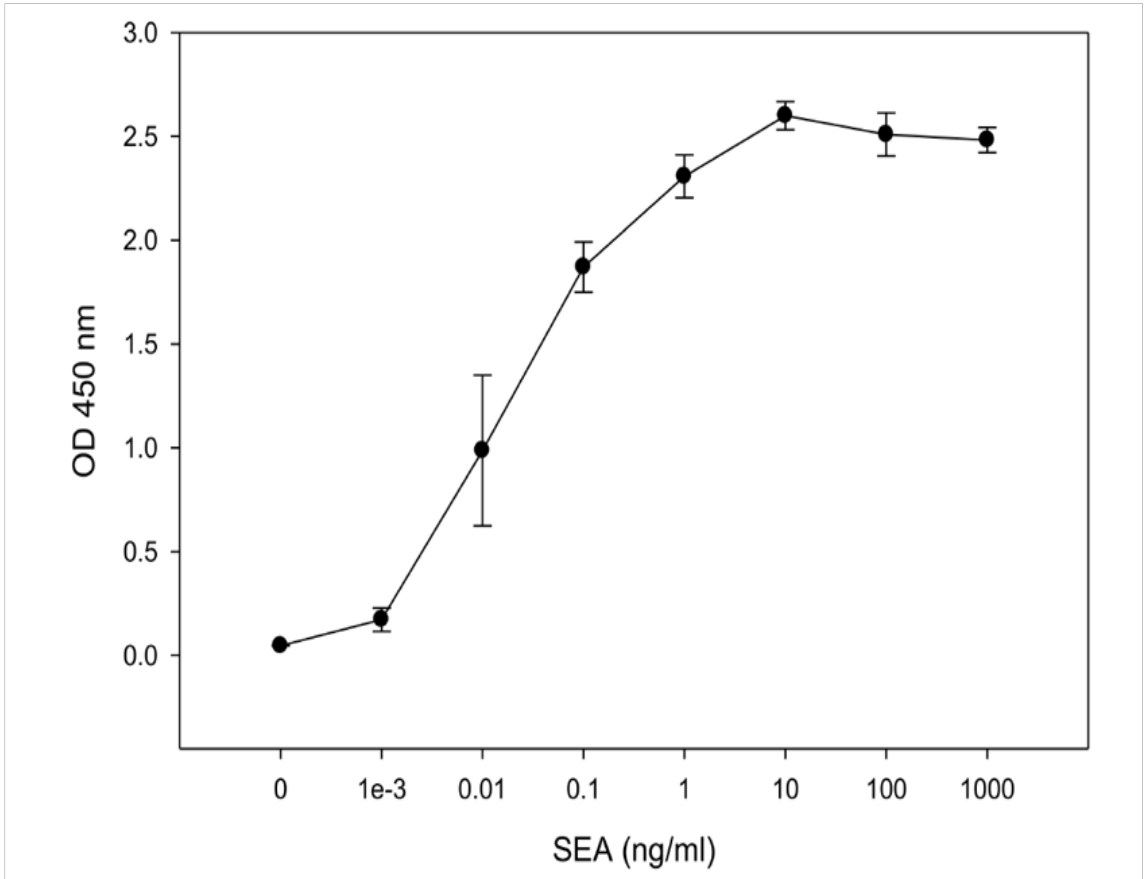
Article

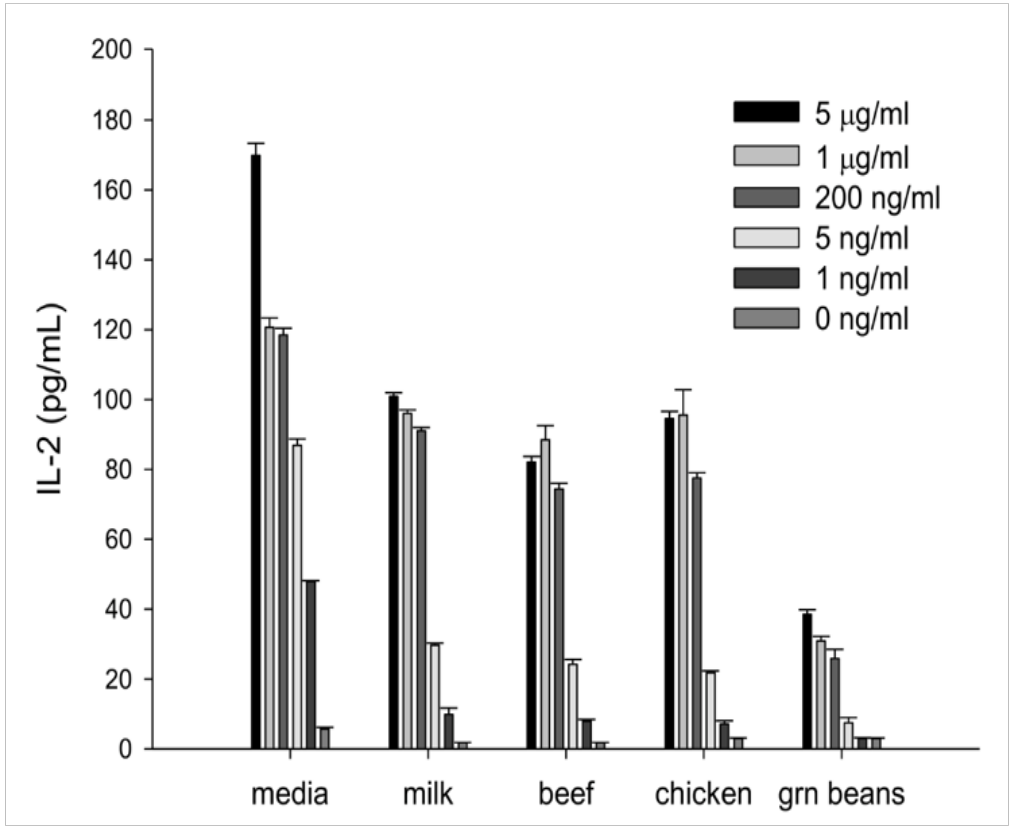
Alternative to Animal Use for Detecting Biologically Active Staphylococcal Enterotoxin Type A

Reuven Rasooly *, Paula Do, Xiaohua He and Bradley Hernlem

T-cell assay for detection of active SEA

LOD: 1 ng/ml or 10^7 time more sensitive than current





Shiga Toxins

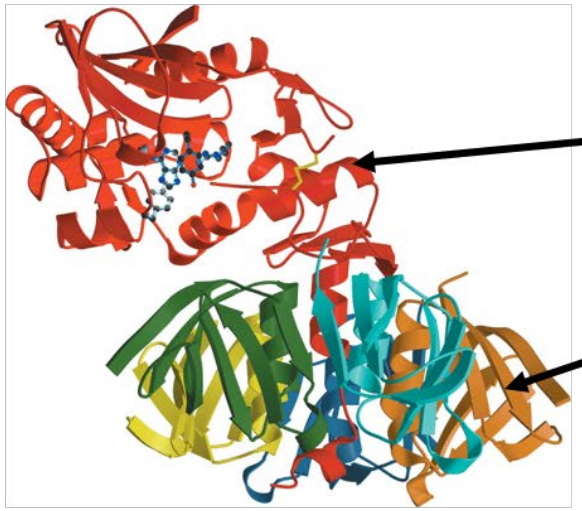
- Develop monoclonal and polyclonal antibodies to shiga toxins
- Compare detection sensitivity of ARS ELISA with current shiga toxin tests
- Detection of low colony forming units (cfu) in ground beef matrix
- Neutralization of shiga toxin +/- presence of antibodies

Challenges for Developing a Stx EIA

Genetic similarity and variation among targets:

STEC Stxs

- Stx1: Stx1a, Stx1c, Stx1d, Stx1e*
- Stx2: Stx2a, 2b, 2c, 2d, 2e, 2f, 2g, 2h* (8 subtypes)



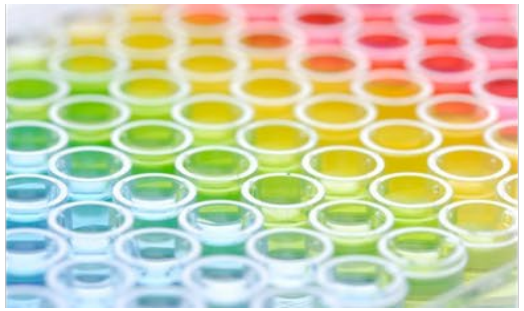
1 A-subunit (32 kDa):
inactivates host protein synthesis

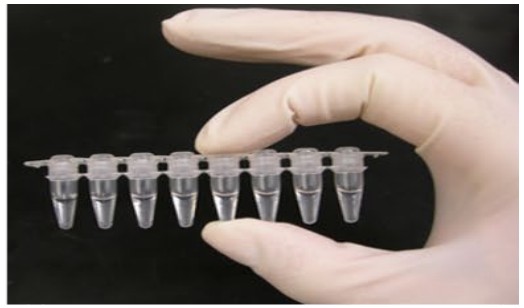
5 B-subunits (7.7kD): binds to
host glycolipid receptors

Fraser, ME et al., Acta Cryst. (2006), F62, 627-630

Stx-based Assays

Assay method	Advantage	Disadvantage
Mouse bioassay	Detects active Stx	Time & labor-intensive,
Vero cell assay		non-specific
PCR	Fast, specific	Detects stx DNA, false negative and positive
EIA	Fast, specific, detects Stxs	Detects both active and non-active Stxs





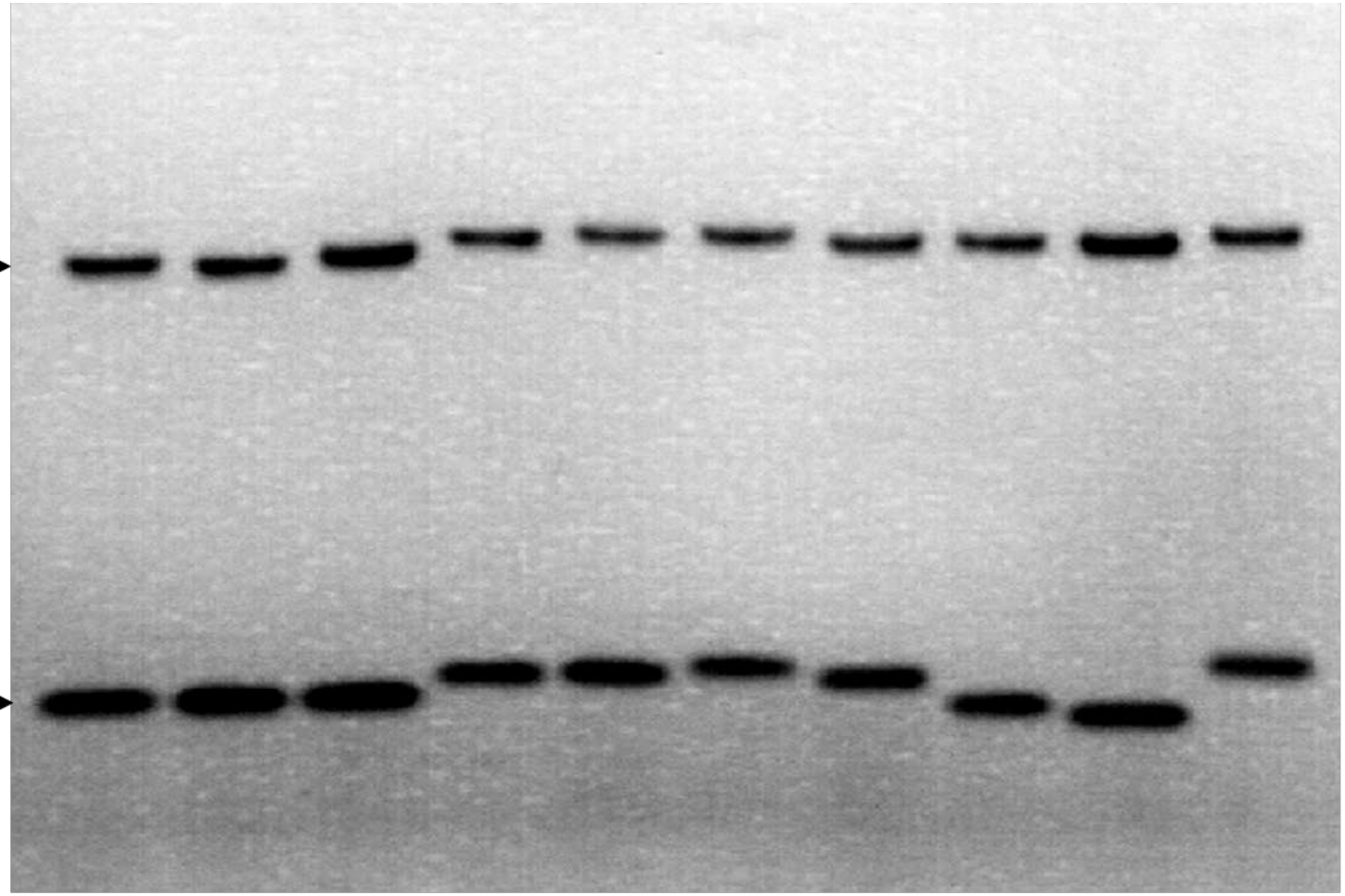
Commercial Stx EIAs Approved by FDA

- Premier EHEC (Microplate EIA)
- ProSpecT Shiga Toxin (Microplate)
- Duopath Verotoxin GLISA (LFD)
- Immunocard STAT!EHEC (LFD)
- BioStar OIA SHIGATOX (Optical EIA)

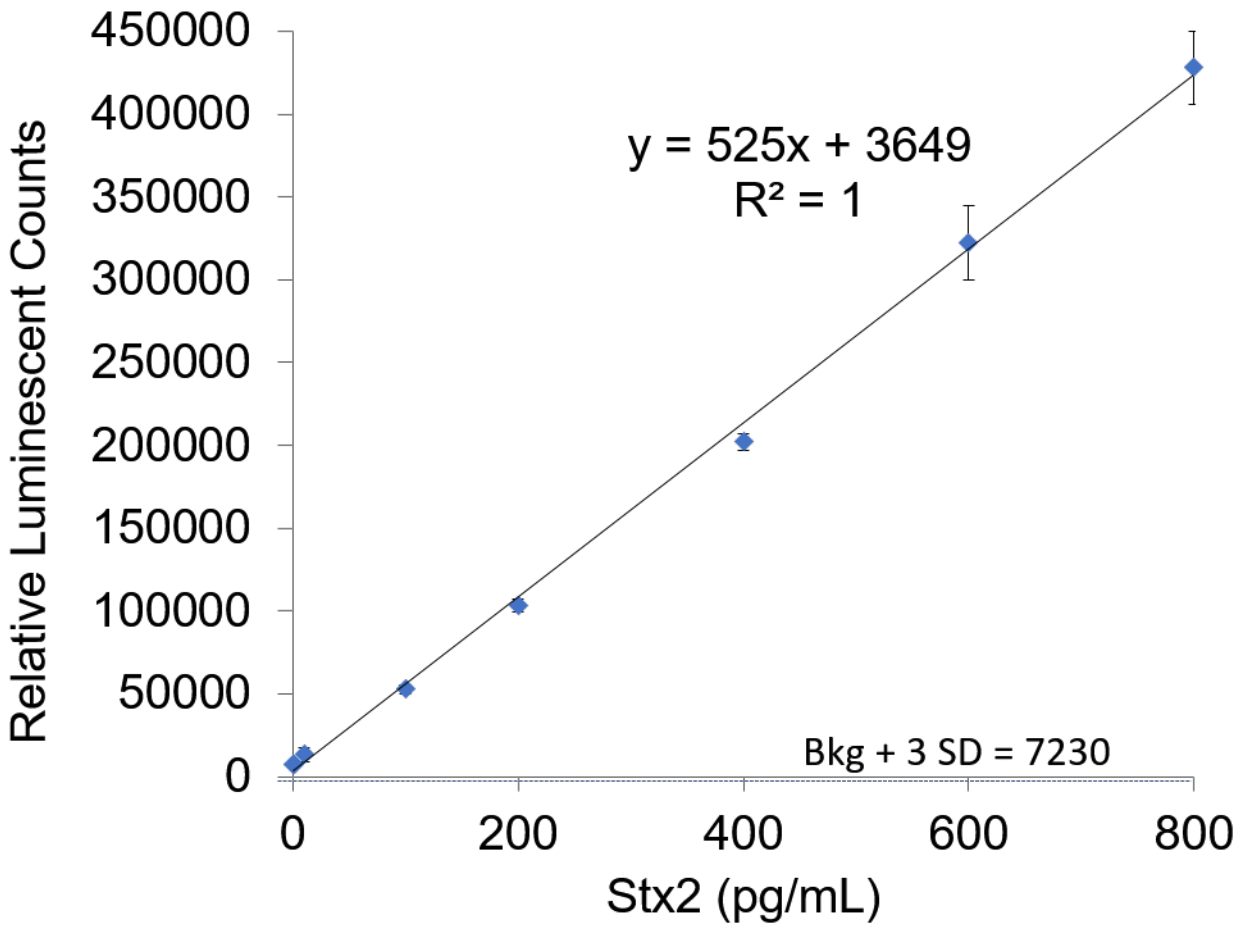
Most current methods do not detect all STEC strains

-CDC MMWR, Oct. 1[illegible], 2009/58(RR12); 1-14

Purified recombinant catalytically inactive shiga toxin (Stx) toxoids



Detection of Shiga Toxin 2a by ELISA



Limit of Detection (LOD)

PBS: 1 pg/mL

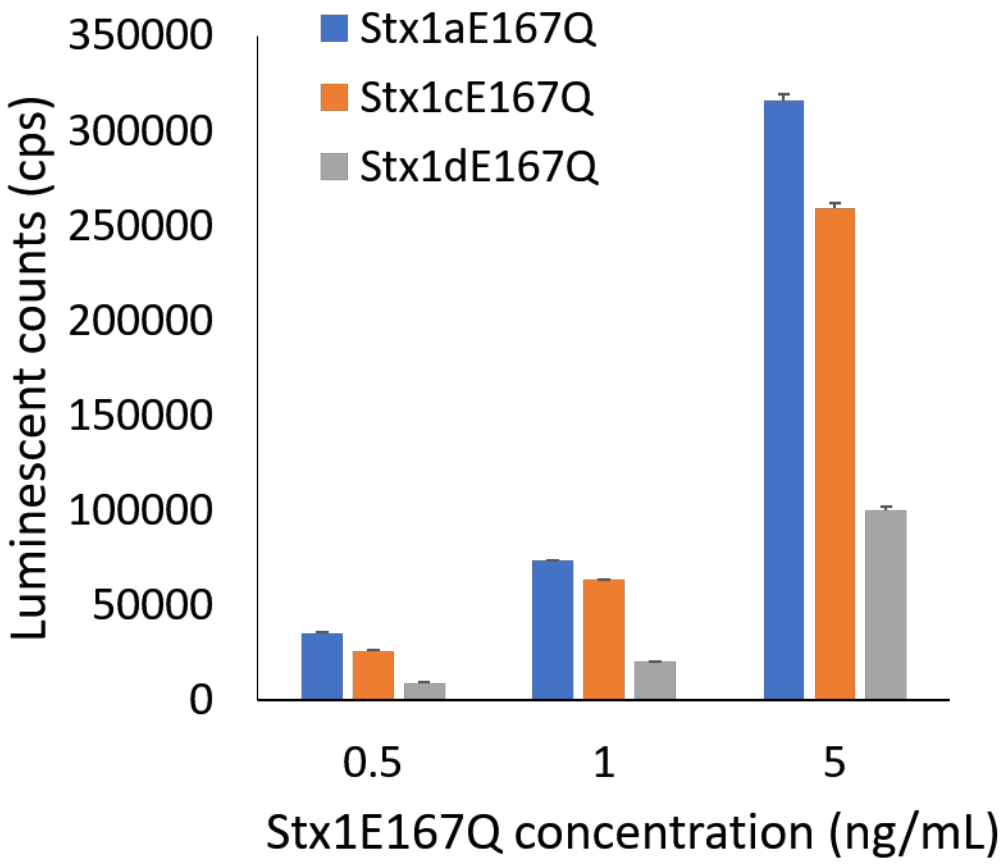
Milk: 1 pg/mL

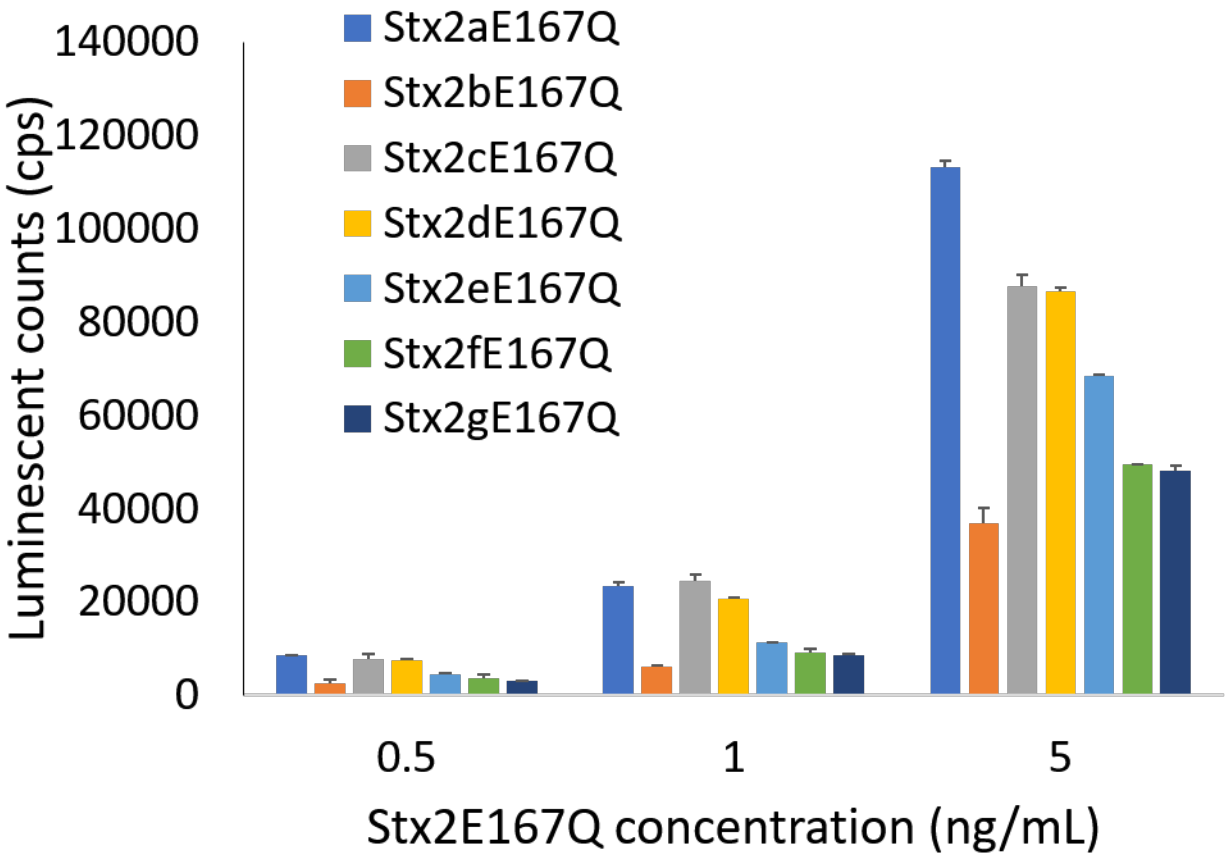
RESEARCH ARTICLE

A New Immunoassay for Detecting All Subtypes of Shiga Toxins Produced by Shiga Toxin-Producing *E. coli* in Ground Beef

Xiaohua He[1]*, Qiulian Kong[2], Stephanie Patfield[1], Craig Skinner[1], Reuven Rasooly[1]

Detection of 10 Subtypes of Stx1 and Stx2 by ELISA





LOD: 10 ~ 50 pg/mL

Comparison of FTDP ELISA with the Meridian Premier EHEC assay

Strain	Serotype	Stx2 sub	ARS ELISA s/n	Stx	Premier s/n	Stx
RM4876	O157:H7	Stx-	1 ± 0	-	1 ± 0	-
RM10638	O157:H7	Stx2a	218 ± 4	++++	50 ± 7	++
RM7005	O118:H12	Stx2b	118 ± 1	++++	3 ± 1	-
RM10058	O157:H7	Stx2c	155 ± 1	++++	51 ± 8	+++
RM8013	ND	Stx2d	139 ± 1	++++	50 ± 8	++
RM7110	O139:NM	Stx2e	9 ± 1	+	2 ± 1	-
RM7007	O128:H2	Stx2f	138 ± 2	++++	48 ± 8	++
RM10468	ND	Stx2g	155 ± 4	++++	2 ± 1	-

Signal/noise ≤ 3: -, 3~10: +, 10 ~50: ++, 50 ~100: +++, >100: ++++

Identification of STEC by Colony ELISA

TSA plate + mitomycin-C

37C ↓ O/N

100 uL TSB + mito-C
37C for 1 h

Strain	Serotype	stx gene	s/n
RM13506	O45	stx1a	1008
RM13508	O103	stx1a	1107
AA1	O174:H8	stx1c, stx2b	476
FF6	O113:H4	stx1c, stx2b	250
RM13149	nd	stx1d	381
RM8082	O121	stx1d	165
RM10638	O157:H7	stx2a	179
RM1913	O157:H7	stx2a	382
RM10058	O157:H7	stx2c	80
MC654	O145:H28	stx1a, stx2c	153
RM8013	nd	stx2d	28
RM7958	O113	stx1a, stx2d	699
RM7988	nd	stx2e	2
RM7007	O128:H2	stx2f	6
CC3	O128:H2	stx2f	4
RM10468	nd	stx2g	28
RM4876	O157:H7	-	1
A TCC25922	O6	-	1

Identification and pathogenomic analysis of an *Escherichia coli* strain producing a novel Shiga toxin 2 subtype

Xiangning Bai[1], Shanshan Fu[1], Ji Zhang[2], Ruyue Fan[1], Yanmei Xu[1], Hui Sun[1], Xiaohua He[3], Jianguo Xu[1,4] & Yanwen Xiong[1,4]

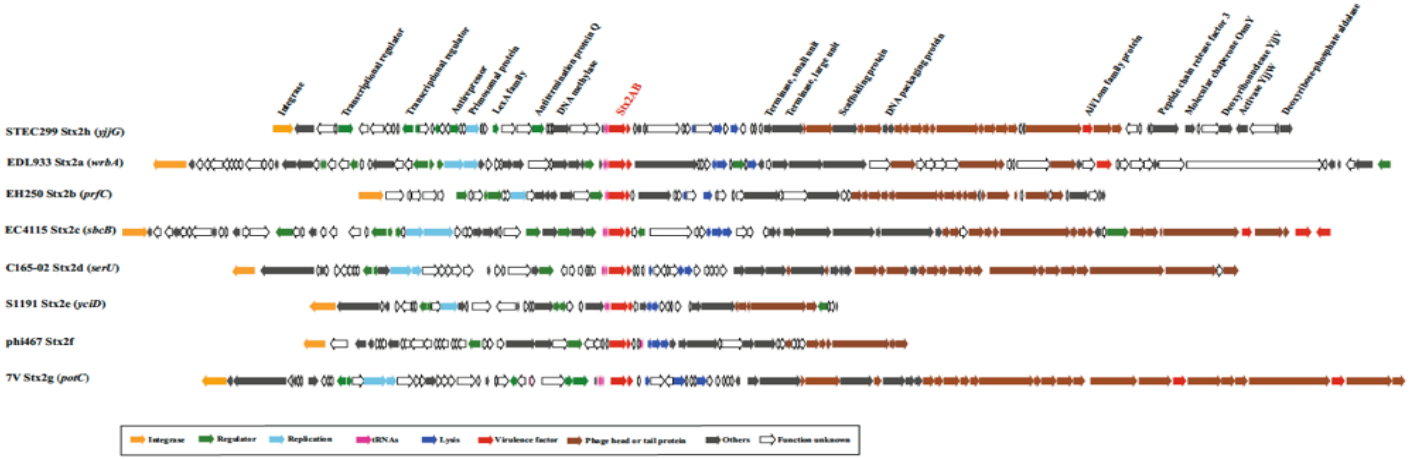
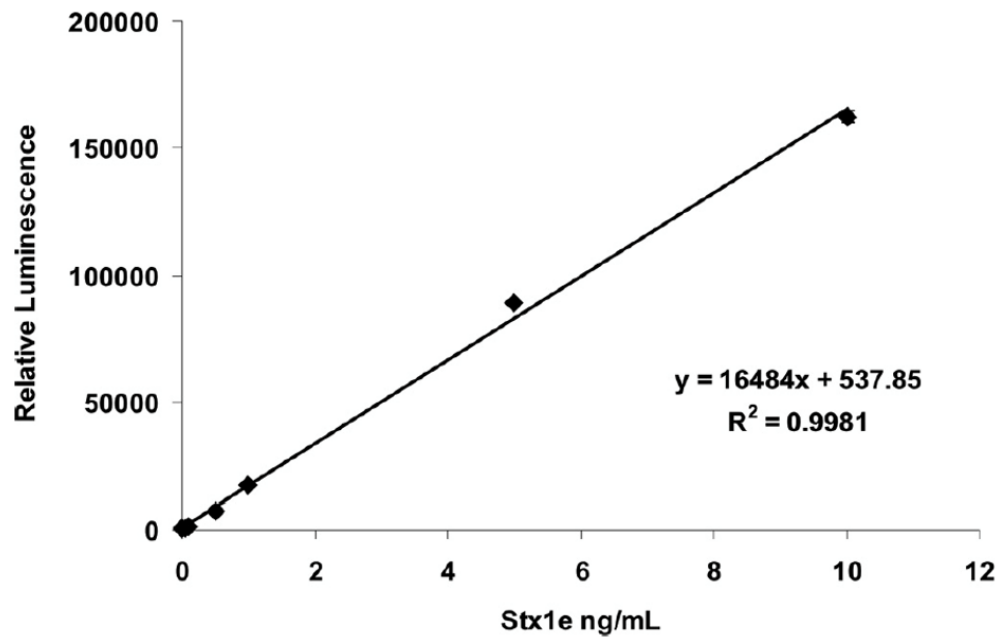


Figure 4. Architecture of Stx2h-converting phages and genomic comparison with other Stx2 phages. The figure shows comprehensive analyses of all Stx2 subtypes converting prophages. Corresponding CDSs are colored as indicated. Integration sites of each phage are presented in parentheses.

New Monoclonal Antibodies against a Novel Subtype of Shiga Toxin 1 Produced by *Enterobacter cloacae* and Their Use in Analysis of Human Serum

Craig Skinner, Stephanie Patfield, Rowaida Khalil,* Qiulian Kong,* Xiaohua He



Phage-mediated Shiga toxin (Stx) horizontal gene transfer and expression in non-Shiga toxigenic *Enterobacter* and *Escherichia coli* strains

Rowaida K.S. Khalil, Craig Skinner, Stephanie Patfield, Xiaohua He

Sensitive detection of active Shiga toxin using low cost CCD based optical detector

Reuven Rasooly [a,*], Josh Balsam [b,c], Bradley J. Hernlem [a], Avraham Rasooly [b,d]

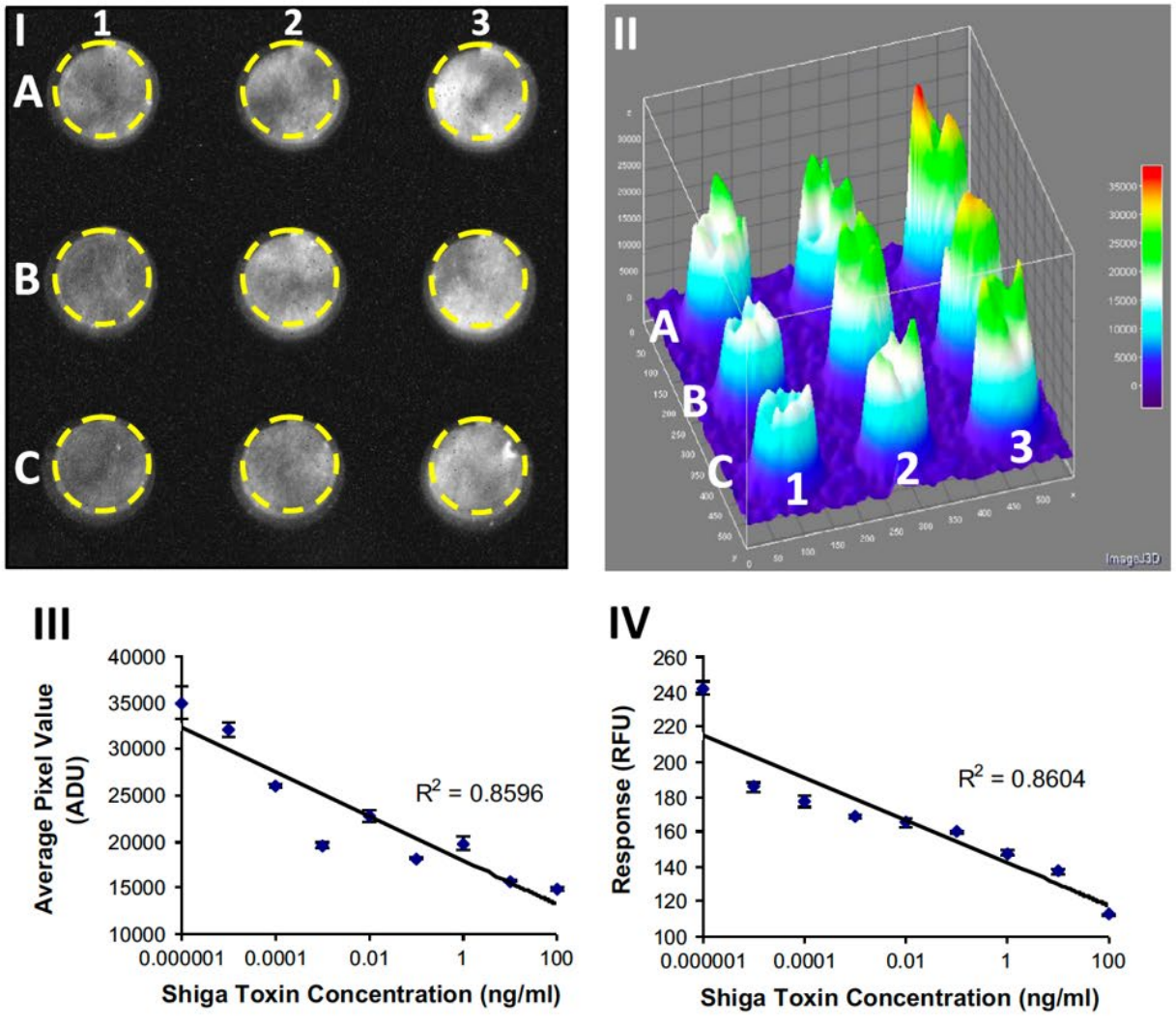
Methods Mol Biol. 2017;1571:233-249. doi: 10.1007/978-1-4939-6848-0_15.

Low-Cost Charged-Coupled Device (CCD) Based Detectors for Shiga Toxins Activity Analysis.

Rasooly R[1], Prickril B[2], Bruck HA[3], Rasooly A[2].

A simple low cost fluorescence detection system was constructed using a CCD camera and light emitting diode (LED) excitation source, to measure Vero cell GFP expression.

The system was evaluated and compared to a commercialfluorometer using photo-multiplier detection for detecting active Stx2 in the range 100 ng/mL–0.01 pg/m



Serum Shiga toxin 2 values in patients during acute phase of diarrhoea-associated haemolytic uraemic syndrome

Xiaohua He[1], Beatriz Quiñones[2], Maroeska Te Loo[3], Sebastian Loos[4], Gaia Scavia[5], Maurizio Brigotti[6], Elena Levtchenko[7], Leo Monnens (leo.monnens@radboudumc.nl)[8]

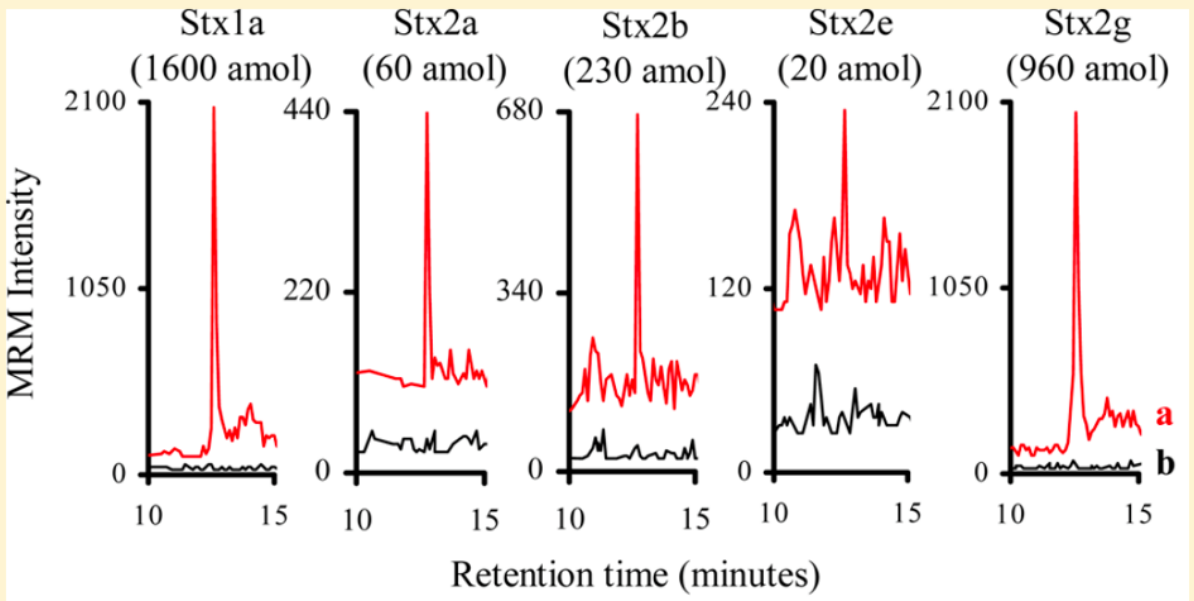
LOD in human sera: 10 pg/ml

Table 1 Clinical characteristics of HUS patients

	Age in years	Gender	Onset diarrhoea presentation	Stx-2 in stool	*E. coli* type	Creatinine mg/dL
1	1.3	Female	9 days	Positive	026	0.67
2	0.8	Male	No diarrhoea	Positive	0121	1.38
3	1.16	Male	6 days	Positive	0157	0.71
4	1.0	Male	6 days	Positive	026	6.00
5	0.7	Male	12 days	Positive	0157	1.11
6	1.7	Male	12 days	Positive	0157	1.54
7	3.4	Male	5 days	Positive	0111	7.27
8	1.0	Male	4 days	Positive	026	0.9
9	0.9	Female	5 days	Positive	0145	2.12
10	0.7	Male	2 days	Positive	026	2.29
11	14.8	Male	5 days	Positive	0104	2.29
12	12.8	Male	2 days	Positive	0104	5.66
13	8.6	Male	2 days	Positive	0104	3.15
14	14.2	Male	6 days	Negative	0104	1.62
15	2.8	Male	6 days	Positive	Negative	1.69
16	2.0	Female	10 days	Positive	0157	5.35
17	8.1	Male	5 days	Positive	0157	7.84
18	4.0	Female	7 days	Positive	0157	8.7

Onset of diarrhoea is defined as days of diarrhoea before presentation with HUS in the hospital.
E. coli types in patients 1–10 were determined by antibody to LPS, by stool culture and serotype analysis in patients 11–14, by stool culture and testing for 0157H7 in patients 15–18.

Mass Spec detection of shiga toxins



Signal intensities corresponding to the amounts of listed Shiga-like toxins. Chromatograms offset for clarity. Sample (a) and background (b)

analytical chemistry
Article
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Safe and Effective Means of Detecting and Quantitating Shiga-Like Toxins in Attomole Amounts

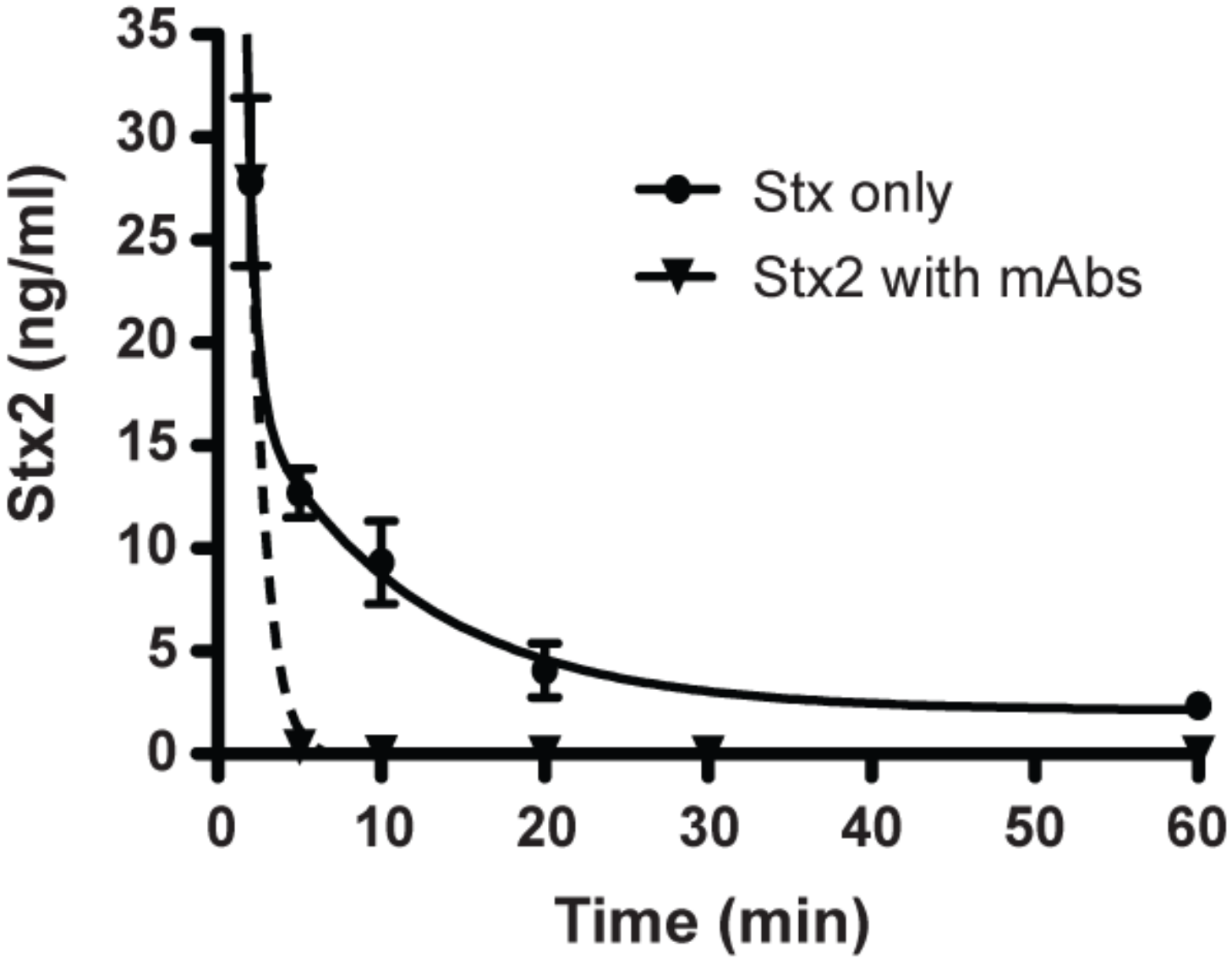
Christopher J. Silva,* Melissa L. Erickson-Beltran, Craig B. Skinner, Irina Dynin, Colleen Hui, Stephanie A. Patfield, John Mark Carter, and Xiaohua He

toxins
MDPI
Article

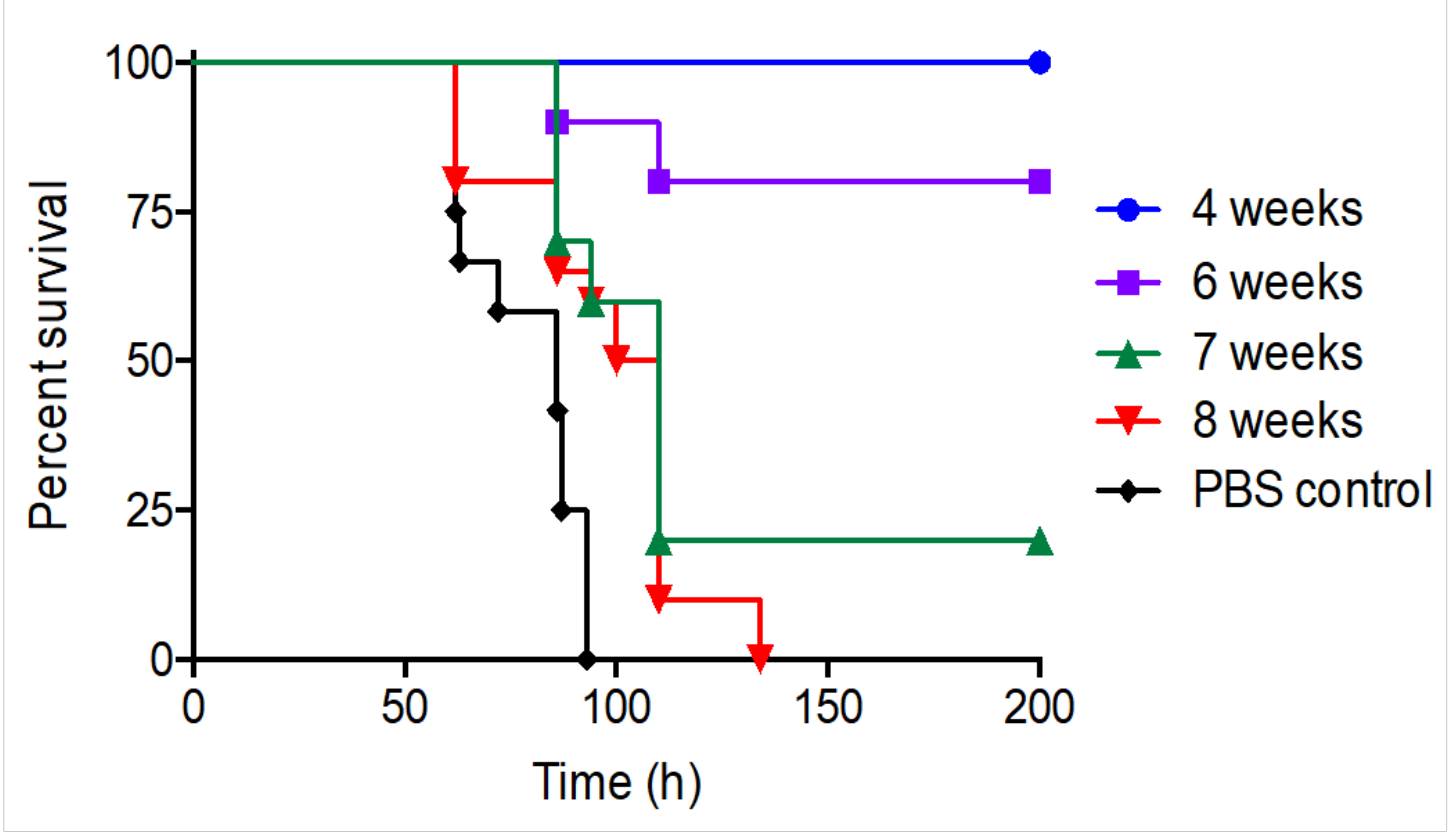
Mass Spectrometry-Based Method of Detecting and Distinguishing Type 1 and Type 2 Shiga-Like Toxins in Human Serum

Christopher J. Silva *, Melissa L. Erickson-Beltran, Craig B. Skinner, Stephanie A. Patfield and Xiaohua He

Stx2 mAbs Clear Toxin from the Mouse Bloodstream



Anti-Stx2 Antibodies Can Protect Mice from Intoxication



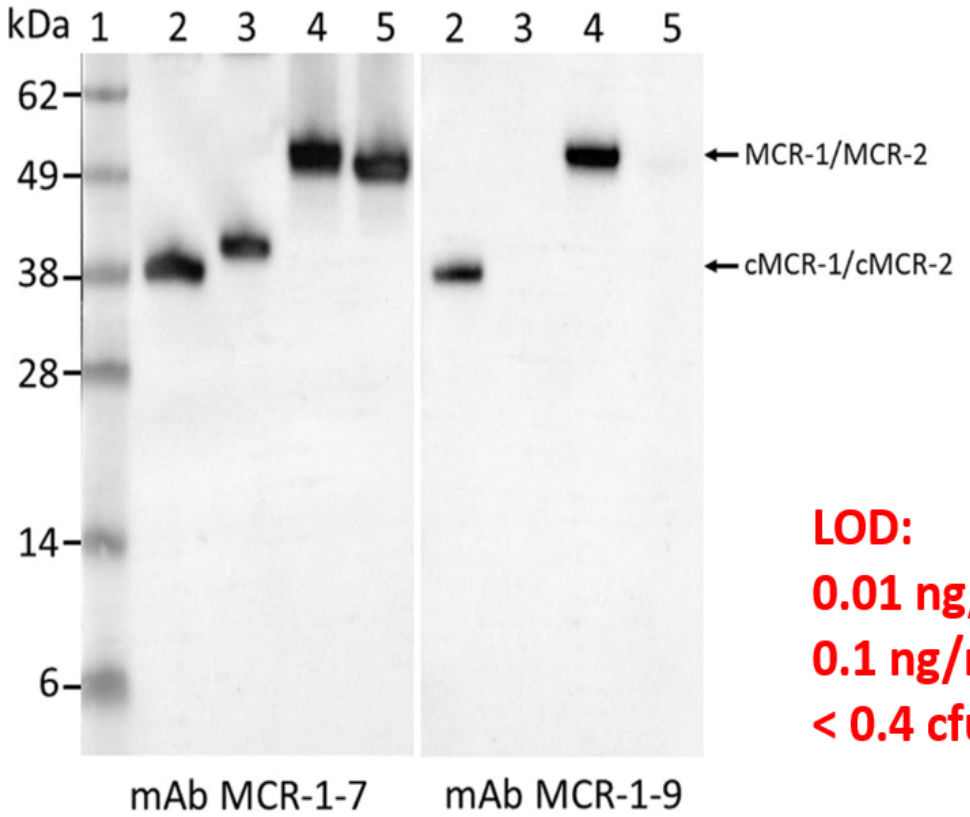
Antibodies given up to 6 weeks prior to intoxication can protect mice from HUS

Antimicrobial Resistance Projects

- Develop mouse monoclonal and rabbit polyclonal antibodies to mcr1 and mcr2 (proteins involved in colistin resistance)
- PCR methods for detection of presence of mcr genes

Development of novel antibodies for detection of mobile colistin-resistant bacteria contaminated in meats

Xiaohua He[1], Daniela Mavrici[1,2], Stephanie Patfield[1] & Fernando M. Rubio[3]

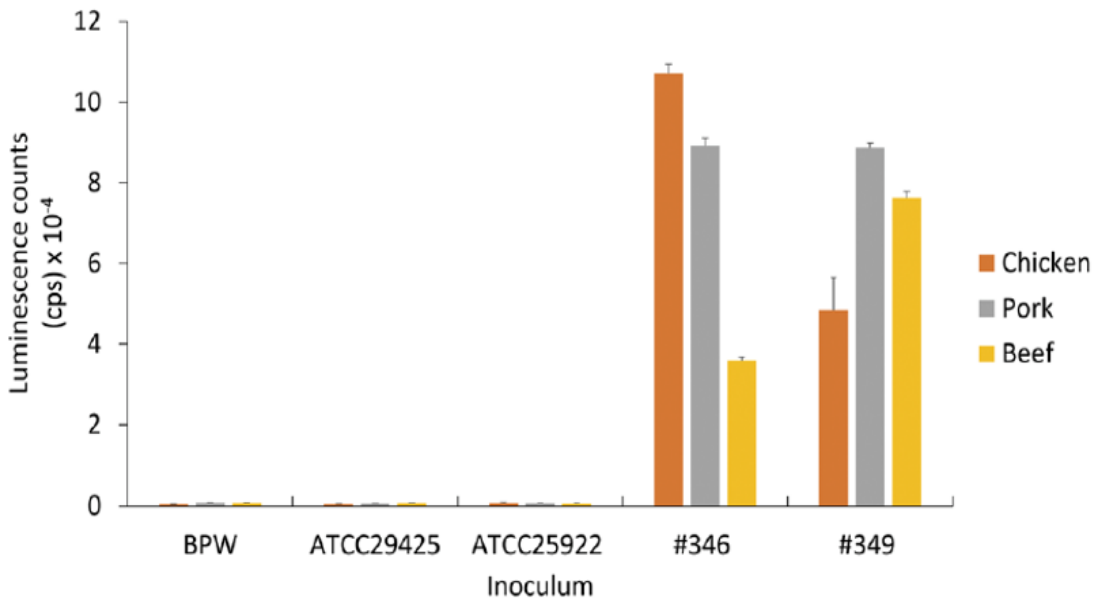


LOD:
0.01 ng/mL for MCR-1
0.1 ng/mL for MCR-2
< 0.4 cfu/g of meat

RESEARCH ARTICLE

Screening for the presence of *mcr-1/mcr-2* genes in Shiga toxin-producing *Escherichia coli* recovered from a major produce-production region in California

Daniela Mavrici[1], Jaszemyn C. Yambao[2], Bertram G. Lee[2], Beatriz Quiñones[2]*, Xiaohua He[1]*



Bacterial strains used to inoculate the food samples

Bacterial strain	Predicted cfu/mL	Actual cfu/mL	mcr-1 gene
BPW	-	-	-
ATCC29425 (mcr-1, -)	10	8.5	-
ATCC25922 (mcr-1, -)	10	9	-
AR-Bank #346 (mcr-1, +)	10	7.3	+
AR-Bank #349 (mcr-1, +)	10	8.8	+

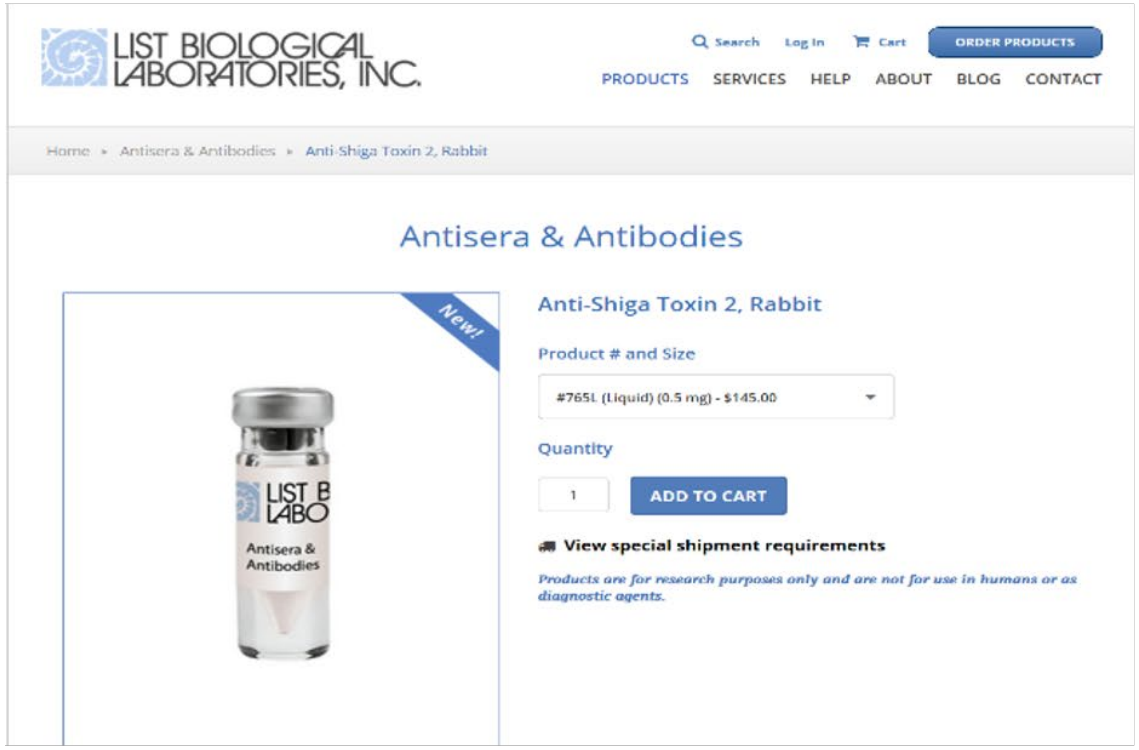
Projects in progress:

Investigation of the prevalence of colistin-resistant bacteria in food

- Cloning, expression and characterization of new MCR variants (mcr-1 to mcr-6)

- Screening samples collected by FSIS from chicken rinse, beef, and pork for the presence of MCR using the immunoassay developed at ARS

Technology Transfer: Immunoassay Kits and Reagents



TechLab Shiga Toxin
Quik Chek Assay

- MTAs (Stx, BoNT, amatoxins, abrin, Mcr)
- 2 patents (2 Stx2, 2f) + 4 pending
- 14 licenses (Abraxis, List Biologicals, Biosentinel TechLab, Allergan, Tetracore, Millipore, etc.) + many pending