Foodborne Toxin Detection and Prevention Research Unit

Western Regional Research Center
Agricultural Research Service
Albany, CA
larry.stanker@ars.usda.gov

Luisa Cheng, RL; Larry Stanker, Candace Bever, Christina Tam

- Advance the Development of Technologies for Detection and Determining the Stability and Bioavailability of Toxins that Impact Food Safety and Food Defense

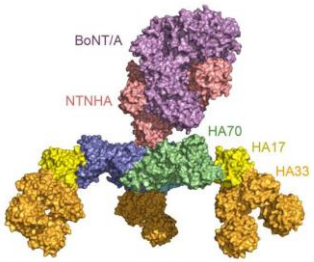
- Bio-control Interventions for High-Value Agricultural Commodities

ARS/FSIS Food Safety Workshop,
NCTC, Shepherdstown, WV
February 21-23, 2018

USDA
United States Department of Agriculture

Advance the Development of Technologies for Detection and Determining the Stability and Bioavailability of Toxins that Impact Food Safety and Food Defense

- Objective 1: Development of structure- and activity-based detection methods for protein toxins.
  - Develop new detection methods for botulinum neurotoxin (BoNT). Serotypes A, B, E and F.
  - Determine the impact of accessory proteins on the detection of BoNTs.
  - Develop activity-based detection methods for staphylococcal enterotoxin (SE).
  - Develop monoclonal antibodies (mAbs) and immunoassays to Shiga toxin (Stx) subtypes and variants, including those from non-E. coli.
- Objective 2: Advance the development of detection methods for non-bacterial toxins.
  - Plant-derived protein toxins such as ricin & abrin.
  - Mushroom toxins such as amatoxins.
- Objective 3: Assess foodborne risks through examination of toxin stability and bioavailability.
  - Use activity-based assays to assess impact of food processing, matrices and accessory proteins on toxin activity.
  - Determine the factors that affect the bioavailability of toxins using rodent bioassay.
- Objective 4: Advance the development of instrumental, portable, and field-deployable testing methods.
  - optical array technologies to detect toxins.
  - instrumental methods to detect toxins based on mass spectra and/or other physicochemical characteristics



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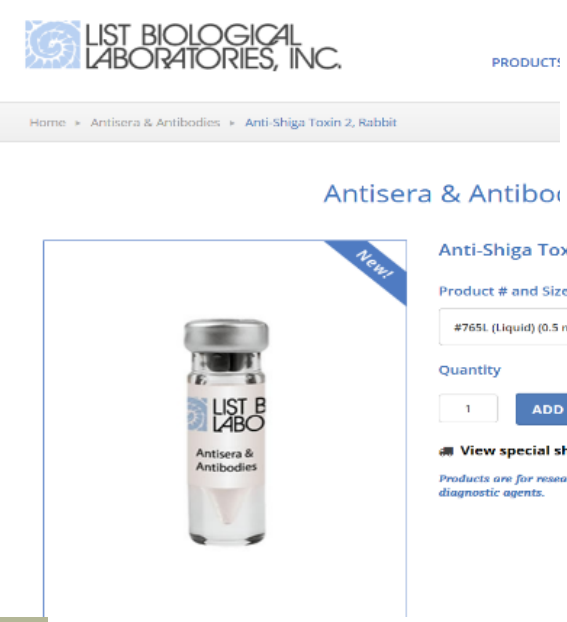
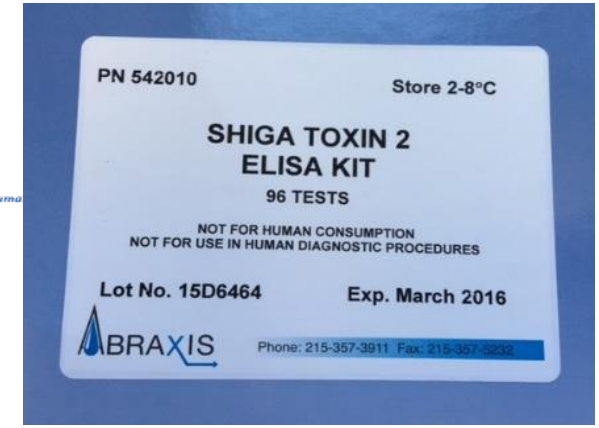
Technology Transfer: Immunoassay Kits and Reagents

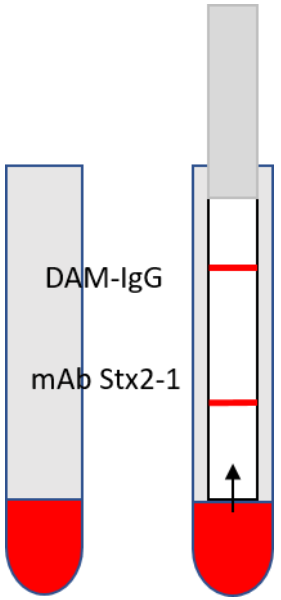
Anti-Botulinum Neurotoxin, Type A (Mouse IgG monoclonal F1-40), Frozen Liquid
Product # and Size
#731L (0.1 mg) - $340.00

Techlab Shiga Toxin Quik Chek Assay

- **6 patents**
- **3 biological materials disclosures**
- **22 licenses**







Detection of **amatoxins** by an immunoassay

Candace Bever
USDA
2.22.2018

Amatoxins are found in Death Cap (*Amanita phalloides)* mushrooms

- Invasive species (Europe to USA)

Amatoxins are found in Death Cap (*Amanita phalloides)* mushrooms

- Invasive species (Europe to USA)
- Confused w/ edible Paddy Straw mushrooms
- Toxicity
  - LD_{50} of ~0.1 mg/kg (Wieland, 1984)
  - ~4 mg amatoxin/mushroom
- RNA polymerase II inhibitor
- No antidote
  - experimental use of IV silibinin/milk thistle

Pringle and Vellinga, 2006


Death Cap


Paddy Straw

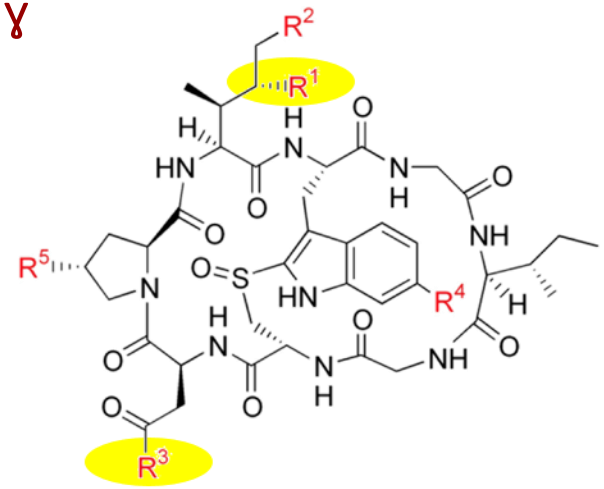
# of mushroom related exposures	Treated in health care facility	Outcome: Moderate	Outcome: Major	Outcome: Death
6,000/yr	2,000	500	30	1-7

Data obtained from American Association of Poison Control Centers annual reports, 2008-2015

Project objectives and goals:

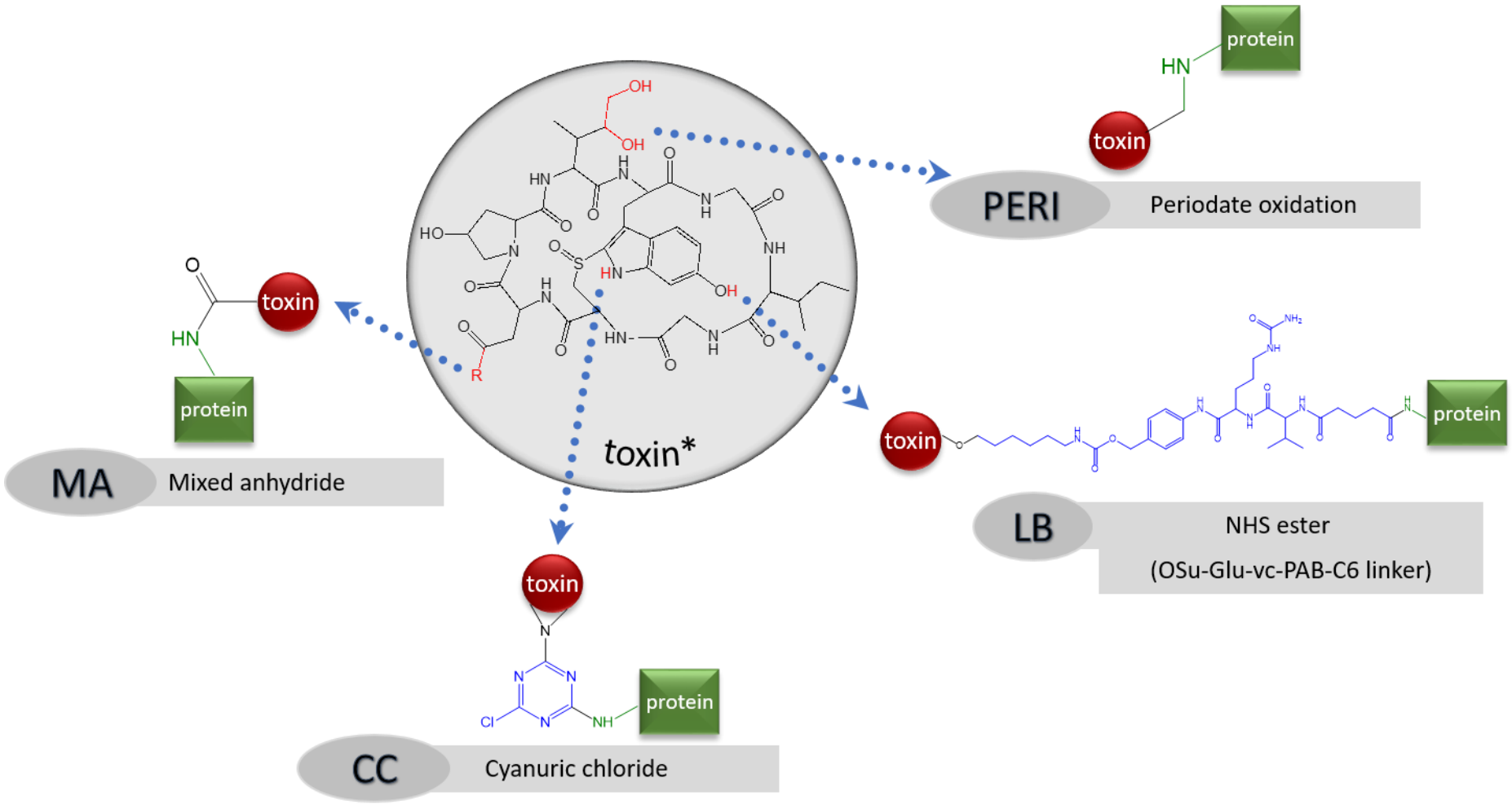
Develop a **rapid**, **sensitive**, and **selective** immunoassay for amatoxins

- Rapid ⇨ antibody-based methods (requires developing antigens/ immunogens)
- Sensitivity ⇨ HPLC ⇨ LOD 10 ng/mL (Enjalbert, et al., 1992)
- Selectivity ⇨ α, β, ɣ



Name	R^1	R^2	R^3	R^4	R^5
α-Amanitin	OH	OH	NH_2	OH	OH
β-Amanitin	OH	OH	OH	OH	OH
γ-Amanitin	H	OH	NH_2	OH	OH
ε-Amanitin	H	OH	OH	OH	OH
Amanullin	H	H	NH_2	OH	OH
Amanullinic acid	H	H	OH	OH	OH
Amaninamide	OH	OH	NH_2	H	OH
Amanin	OH	OH	OH	H	OH
Proamanullin	H	H	NH_2	OH	H

Antigen/Immunogen synthesis



Assay characterization: sensitivity

Antibody Activity

-------coating antigen-------

ID imm	cc	ma	peri	LB
63 cc	+++	--	--	--
64 cc	+++	--	--	--
65 ma	+	+	--	--
66 ma	--	+	--	--
53 peri	+	+	+	+
54 peri	+	+	+	+
57 LB	+	+	++	+++
58 LB	+	+	++	+++

------- rabbit sera -------

Assay Detection (IC_{50} ng/mL)

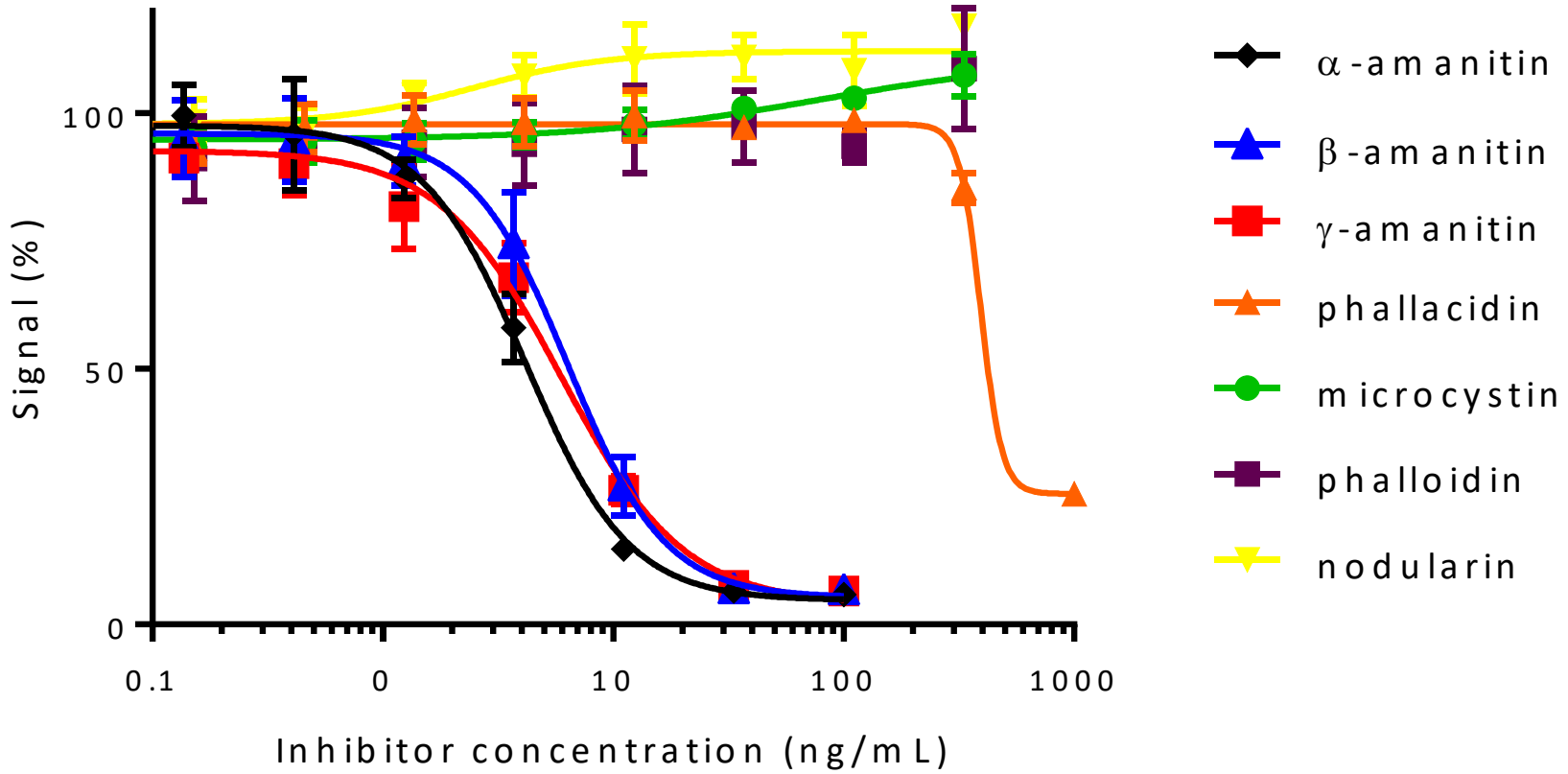
-------coating antigen-------

ID imm	cc	ma	peri	LB
63 cc	>1000	--	--	--
64 cc	>1000	--	--	--
65 ma	>1000	>1000	--	--
66 ma	--	>1000	--	--
53 peri	0.06	0.28	0.19	2.98
54 peri	0.1	0.12	0.13	0.42
57 LB	>100	8.65	0.47	>1000
58 LB	0.48	5.97	0.07	>1000

------- rabbit sera -------

Identified suitable pairs of sera and coating antigen reagents that give great sensitivity

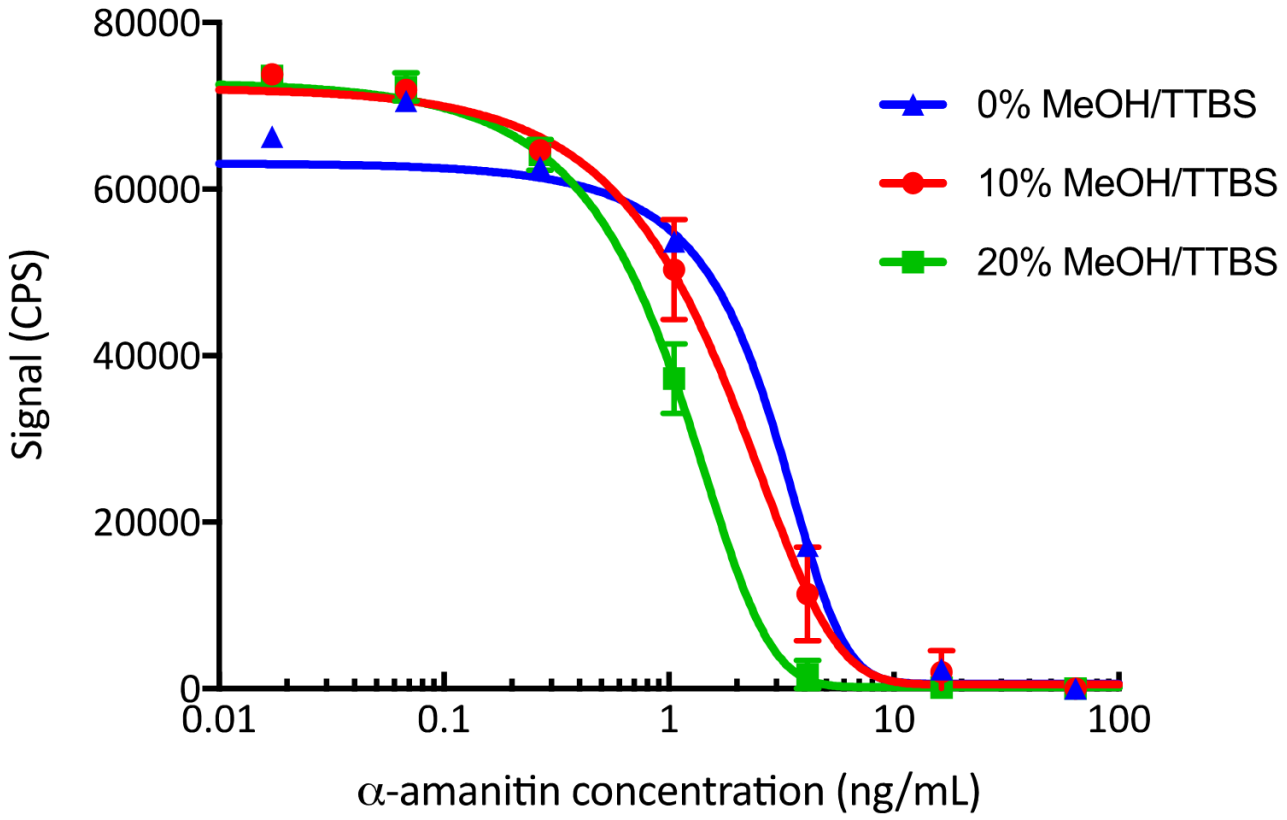
Assay characterization: selectivity



Assay is selective for α-, β-, ɣ-andamatoxins

Rabbit #58, plate coated with BSA-ama-peri @ 0.1 ug/mL

Assay characterization: matrix effects (MeOH)



Assay is minimally impacted by methanol

Rabbit #58, plate coated with BSA-ama-peri @ 0.1 ug/mL

Sample analysis: methods

A. phalloides

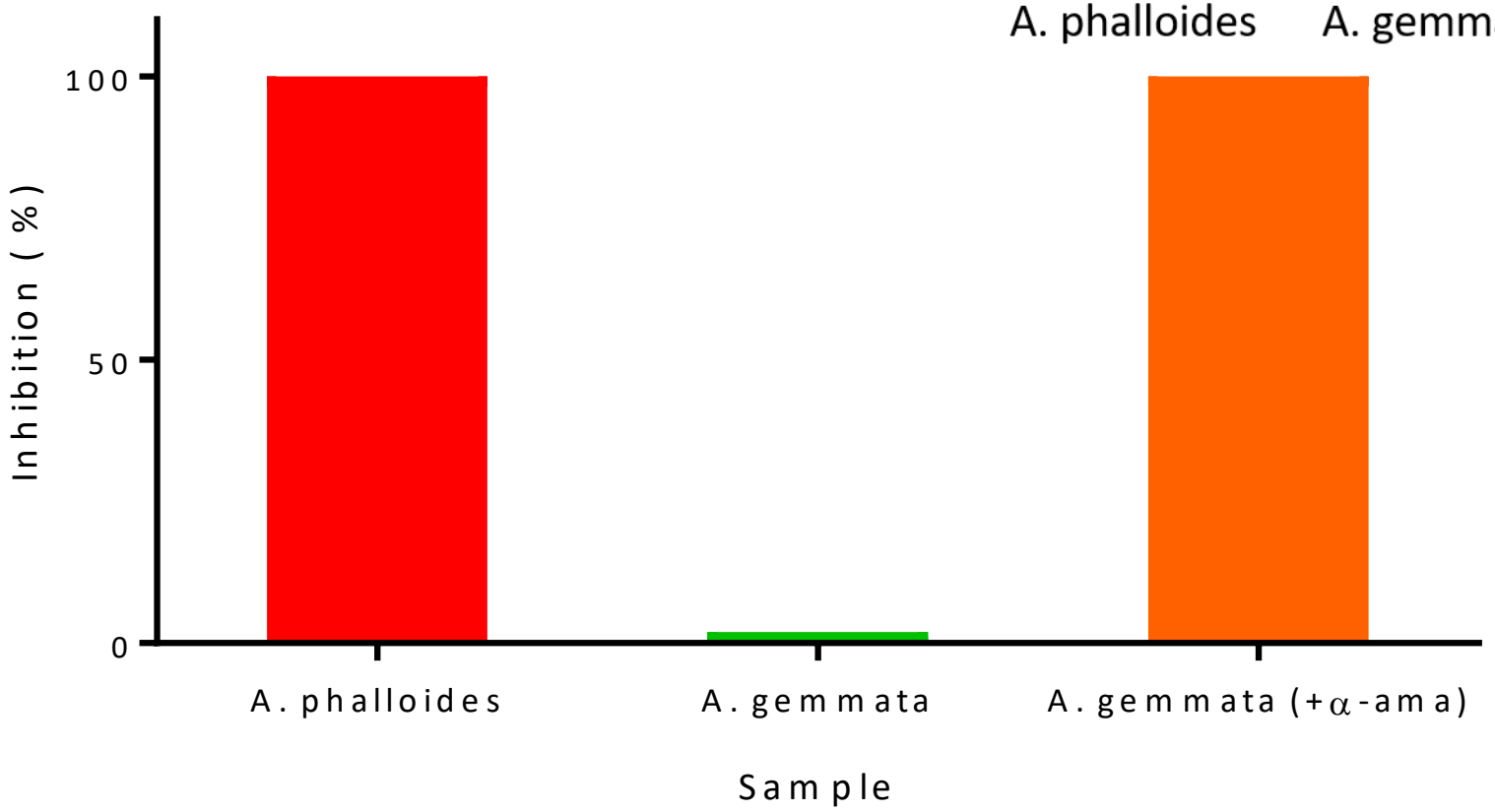
A. gemmata

- Mushrooms collected from a National Park
- Dried overnight in oven
- ~300 mg of dried mushroom (cap) ground to a powder
- Toxin extracted with ~3 mL of MeOH+water+0.01 N HCl (8:1:1, v/v/v)
- Extract diluted 1:100 with assay buffer and directly analyzed

Sample analysis: results

A. phalloides

A. gemmata

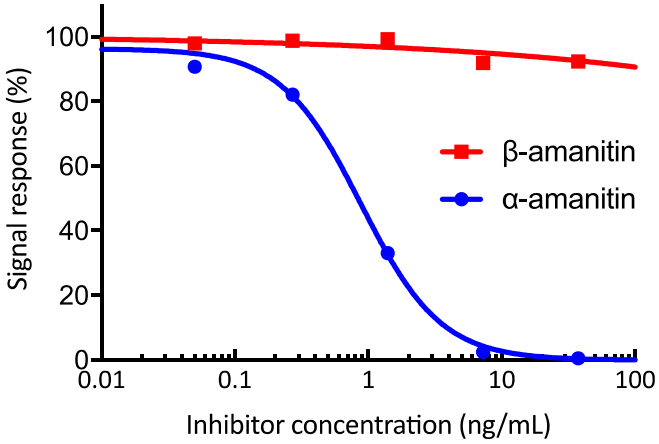


Summary and future work

- Immunogen synthesis appears to be successfully inducing antibody responses
- Assay has great sensitivity (ng/mL)
- Assay (rabbit sera) detects amatoxins (α, β, and ɣ)
  - Mouse mAbs underway and might demonstrate α v. β selectivity

Future work….

- Validate assay with further sample analysis
- Transfer immunoreagents to rapid and portable formats
- mAb development



Abrin toxin detection and inactivation

Christina Tam

USDA-ARS

02-22-18

Abrin is a highly toxic plant toxin

- Found in the seeds of the rosary pea (or jequirity pea)*, Abrus precatorius*
- A Select Agent and a bio-terror threat to our food supply similar to ricin
- Abrin is an A-B chain toxin that deadenylates rRNA to inhibit protein translation
- No antidote

Abrin Toxicity

Species	Route	Lethal Dose (µg/kg)
Human (70 kg)	iv oral	> 0.3 10-1000
Mouse (20 g)	iv ip oral	0.7 2-20 5-20 x 10^3

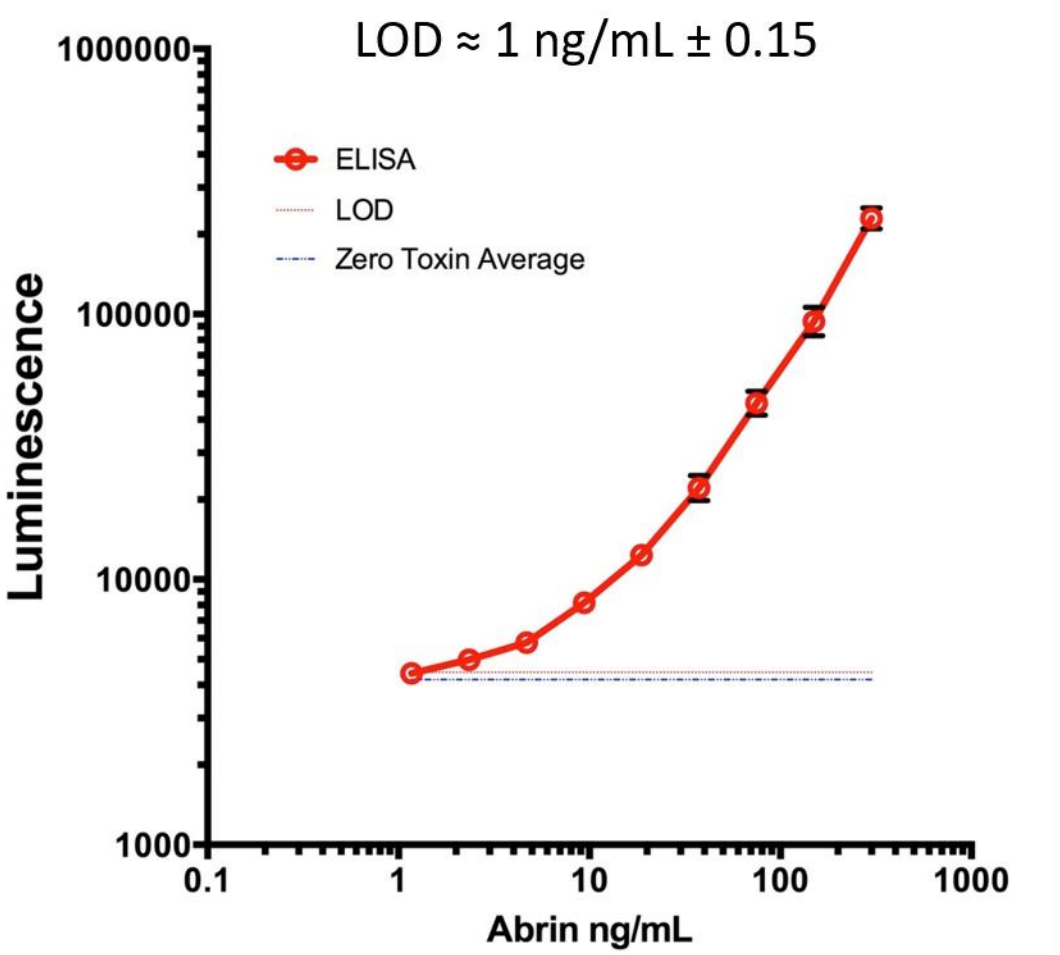
Abrin is 31.4 times more toxic than ricin for mice i.v.

Development of a monoclonal-monoclonal capture ELISA for abrin

Abrin monoclonal antibodies

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

A capture ELISA for abrin



Detection of abrin in food matrices

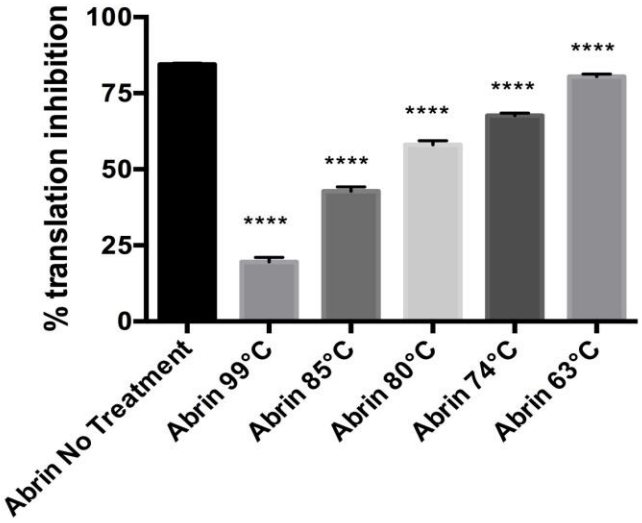
Food Matrix	LOD
Whole milk	20 ng/mL
2% milk	5 ng/mL
Non-fat milk	5 ng/mL
Orange juice	40 ng/mL
Apple juice	10 ng/mL
Carrot juice	4 ng/mL

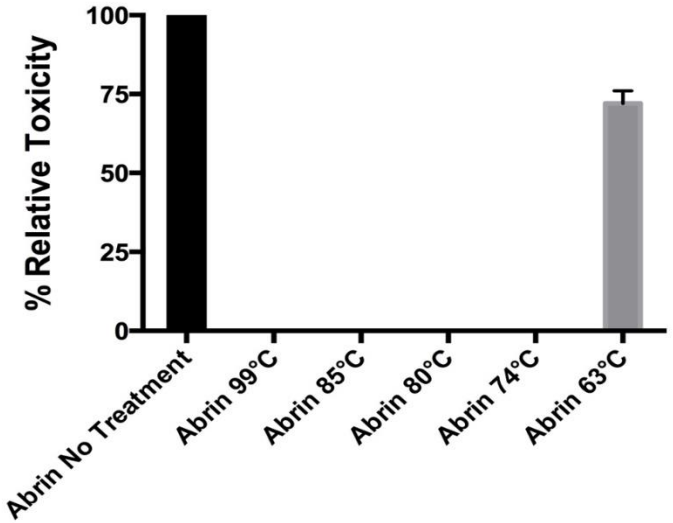
Abrin Bioavailability and Toxicity

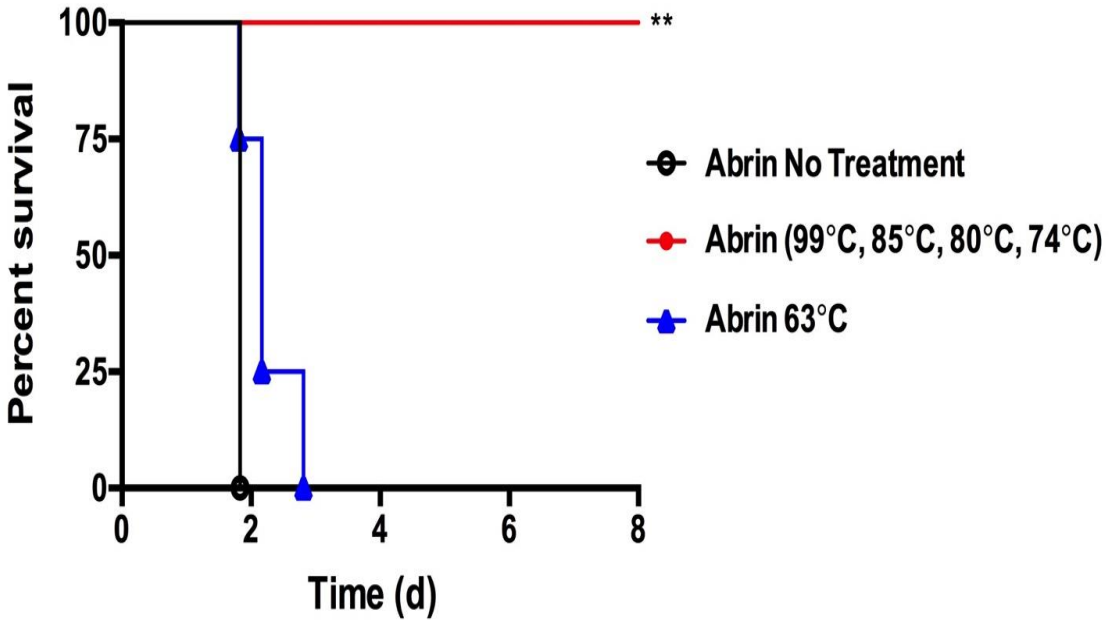
Abrin inactivation conditions

- Temperature inactivation of abrin from 63°C to 99°C using a thermocycler PCR
- pH treatment between pH 2.0 and pH 9.0 for 1 h at RT then neutralization to pH 7.0
- 3 assays were used to determine toxin inactivation:
  - In vitro cell free translation
  - In vitro Vero cell cytotoxicity
  - In vivo mouse bioassay

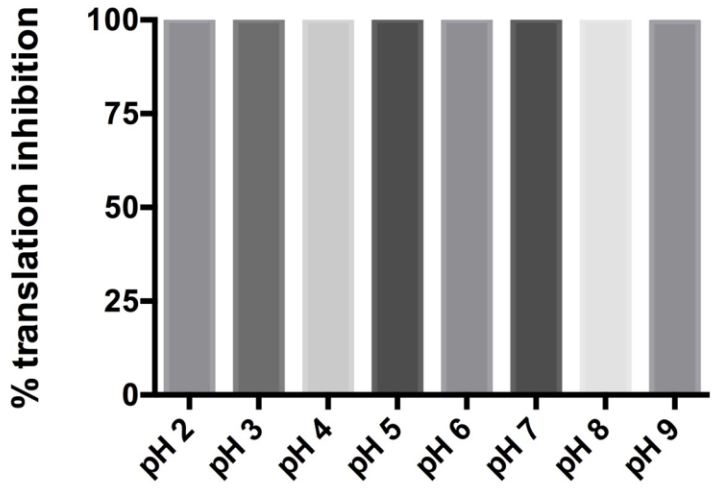
Abrin inactivation requires temperatures ≥ 74°C

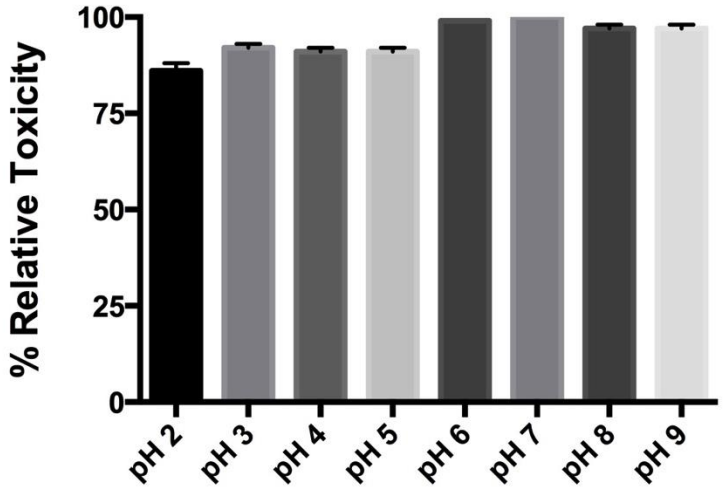


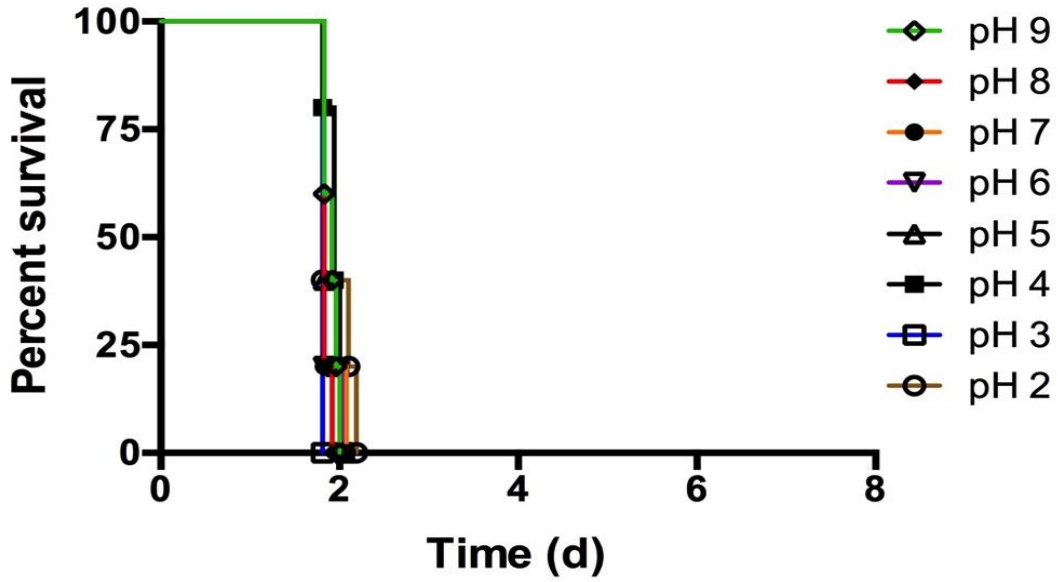




pH treatment has no effect on abrin toxicity







Conclusions

- A capture ELISA for Abrin was developed with an LOD ≈ 1 ng/mL
- Abrin can be detected in food matrices using the ELISA
- Functional inactivation of abrin requires temperatures ≥ 74°C
- Abrin function is not affected by exposure to pH
- Assays to validate toxin inactivation must be carefully chosen to reflect actual function

Future Directions

- Optimization of ELISA to detect abrin in multiple economically important foods
- Inactivation of toxin in complex food matrices, validation using the 3 assays, and detection using ELISA
- Development of new and improved abrin antibodies using various technologies

Thank you

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