Evolution of Mass Spectrometry in Laboratory Testing of Biothreat Agents

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Abstract

Clinical, agricultural and public health laboratories (PHL) screen thousands of samples daily for bacterial agents and toxins. Sample preparation, processing and analysis using conventional microbiological methods can range from days to weeks for pathogen identification. Two agents of high concern are Botulinum neurotoxin (BoNT) which cause the disease known as botulism by inhibiting neurotransmitter release at the neuromuscular junction and ricin toxin which inhibits protein synthesis.

Both methods require extensive and cumbersome processing leading to time consuming testing as negative results can take up to four days to identify. As the number of specimens has increased in laboratories, there exists a great need to quickly identify sources of exposure which has led our laboratory to explore the use of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF/MS).

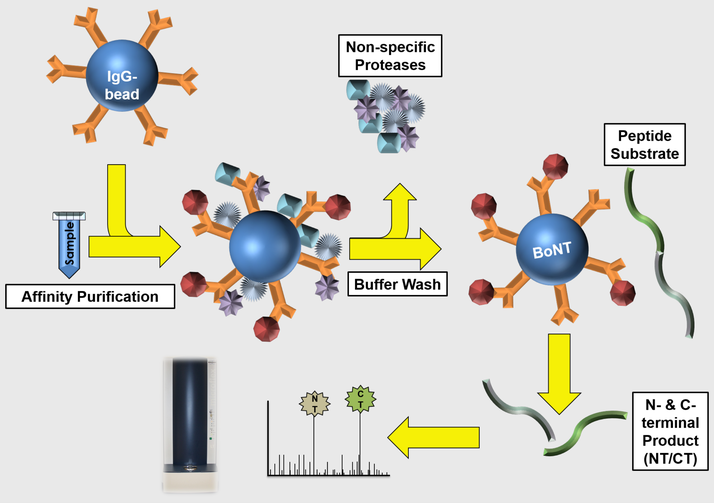
An endopeptidase-based mass spectrometry method, which was developed by the CDC, has been validated with the Bruker Daltonics MALDI-TOF Biotyper. This MS method utilizes the endoproteinase activity of the toxin to identify all BoNT types (A-G) with a MALDI-TOF/MS by cleaving peptides at specific sites. Each toxin is identified by the mass-to-charge ratios of these fragmented peptides for BoNT and the depurination of a RNA substrate for ricin toxin.

For each assay, BoNT and ricin toxins were spiked into over ten different food matrices to assess detection capability. Assay sensitivity for BoNT/A, /B, /E, and /F ranged from 0.3 to 25 MLD_{50}. Active ricin toxin was detected as low as 0.5 ng.

Purpose

To identify and validate a method that is capable of detection BoNT and ricin toxin in food matrices without requiring the use of animals and costly traditional methods.

BoNT Mass Spectrometry Method



Peptide Substrate Sequences

Table 1: BoNT/A, /B, /E, and /F peptide substrates with respective cleavage products

Peptide Substrate	Sequence	Observed m/z	Cleavage 1 m/z	Cleavage 2 m/z
BoNT/A*	Ac-RGSNKPKIDAGN**QR**ATRXLGGR-NH_2	2406	998	1426
BoNT/B	LSELDDRADALQAGAS**QF**ESSAAKLYK RKYWWKNLK	4026	1759	2283
BoNT/E	WWWAKLGQEIDTRNRQKD**(hR)I**MAKA DSNKR-NH_2	3615	1132	2500
BoNT/F	TSNRRLQQTQAQVDEVVDIMRVNVDKV LERD**QK**LSELDDRADAL	5111	1345	3783

Assay Limit of Detection and Spiking Studies

- Eleven different matrices, including over 20 different types of chicken, turkey and pork, were spiked at three concentrations of BoNT/A, /B, /E, /F.

Table 2: BoNT MS assay LOD and food spike concentrations

BoNT Type	LOD (MLD_{50})	Low (MLD_{50})	Medium (MLD_{50})	High (MLD_{50})
A	18	180	360	540
B	25	250	500	750
E	0.3	3	6	9
F	9	88	176	264

Table 3: BoNT/A, /B, /E, and /F matrix spiking results

Matrix	BoNT/A	BoNT/B	BoNT/E	BoNT/F
Chicken (breast, wings, thighs)	Positive	Positive	Positive	Positive
Pork (chop, loin, sausage)	Positive	Positive	Positive	Positive
Kielbasa	Positive	Positive	Positive	Positive
Pepperoni	Positive	Positive	Positive	Positive
Smoked Honey Deli Ham	Positive	Positive	Positive	Positive
Hot Italian Sausage	Positive	Positive	Positive	Positive
Chicken Egg Rolls	Positive	Positive	Positive	Positive
Infant Formula	Positive	Positive	Positive	Positive
Turkey (bacon, sausage, ground)	Positive	Positive	Positive	Positive
Egg Whites	Positive	Positive	Positive	Positive
Ground Lamb	Positive	Positive	Positive	Positive

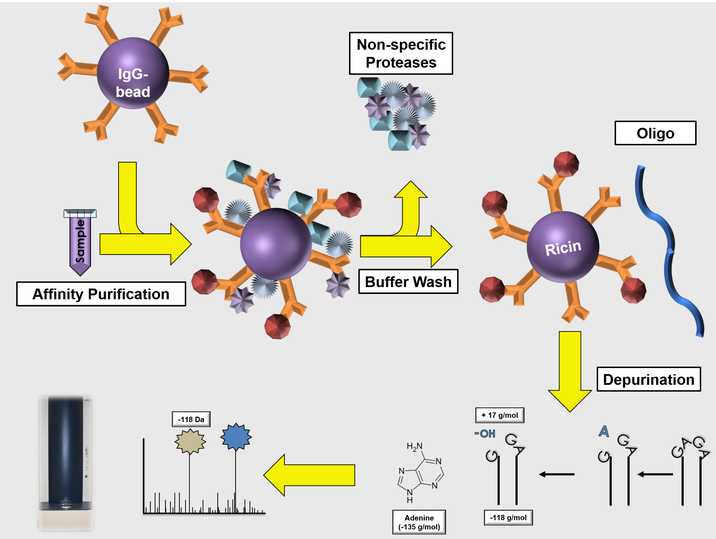
BoNT MS Assay – PHL Food Specimens

Table 4: Food specimens received at the Wadsworth Center suspected of containing BoNT. Specimens were tested by PCR, MS assay, and the mouse bioassay (when available).

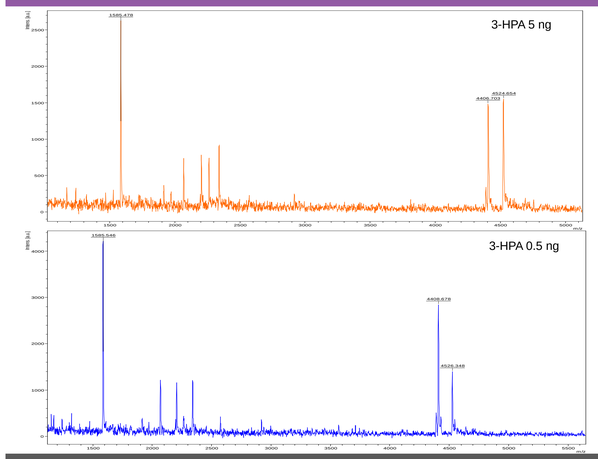
Specimen ID	Specimen Type	PCR Results	BoNT Assay Results (ABEF)	Mouse Bioassay Results
12-08991	Tofu	Positive, B	Positive, B	Positive, B
14-18209	Spaghetti Sauce with Peas and Meat	Positive, A & B	Positive, A	Positive A
14-18198	Spaghetti Sauce	Negative	Negative	N/A
16ENV57	Infant Formula	Negative	Negative	N/A

- The BoNT MS assay has shown its utility for multiple food specimens.
- In one instance, a tofu specimen that was received at the Wadsworth Center suspected of containing BoNT was tested by three assays: PCR, MS, and the mouse bioassay.
- *Clostridium botulinum* type B was successfully identified by PCR and confirmed of containing BoNT/B by the mouse bioassay.
- The sample was re-tested by the MS assay which positively identified the specimen as containing BoNT/B.
- Beginning in 2015, the mouse bioassay was no longer conducted at the Wadsworth Center and instead BoNT is strictly identified by the MS assay.

Ricin Mass Spectrometry Method



Ricin MS Assay LOD



- The LOD of the Ricin MS assay in PBS was determined to be 0.5 ng.
- The LOD was determined to be 0.5 ng in infant formula.
- Preliminary data suggest the LOD in ground meat to be 5 ng.

Conclusion

- The MS assay LOD for detection of BoNT/A, /B, /E, and /F ranged from 0.3 to 25 MLD_{50}.
- The MS assay LOD for detection of ricin toxin was 0.5 ng.
- When the MS assay is utilized in conjunction with the Wadsworth Center developed PCR assay, the time required for BoNT identification is dramatically reduced from 4 days to 1 day.
- Identification of BoNT and ricin toxin in multiple food matrices using mass spectrometry-based methods can replace the need for performing traditionally costly, time-consuming methods.

Acknowledgement

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