

DETECTION OF BOTULINUM TOXIN SUBTYPES IN FOOD MATRICES

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ABSTRACT: Botulinum toxin is one of the most toxic compounds with no effective chemical antidote. There is a significant threat that it may be bioweaponized. Preventive measures for deliberate botulinum toxin food contamination is a growing concern. The goal of this research project is to evaluate the Food Biological Agent Detection Sensor Program (FBADS) system for immunodetection of botulinum toxin in a variety of food matrices. The FBADS system being tested for its limits of detection and sensitivity are the Meso Scale Diagnostics (Gaithersburg, Maryland) Model PR2 1900 and its botulinum assay kit for toxin subtype -A. The suitability of this kit was evaluated on a wide variety of food matrices. The detection and identification of the targeted antigens under realistic conditions in this assay will be compared to the diagnostic ability of standard ELISA. This assay is simple to use and requires minimal training and if can be extended to apply to a variety of food matrices can prove very effective tool in the event of intentional or accidental contamination.

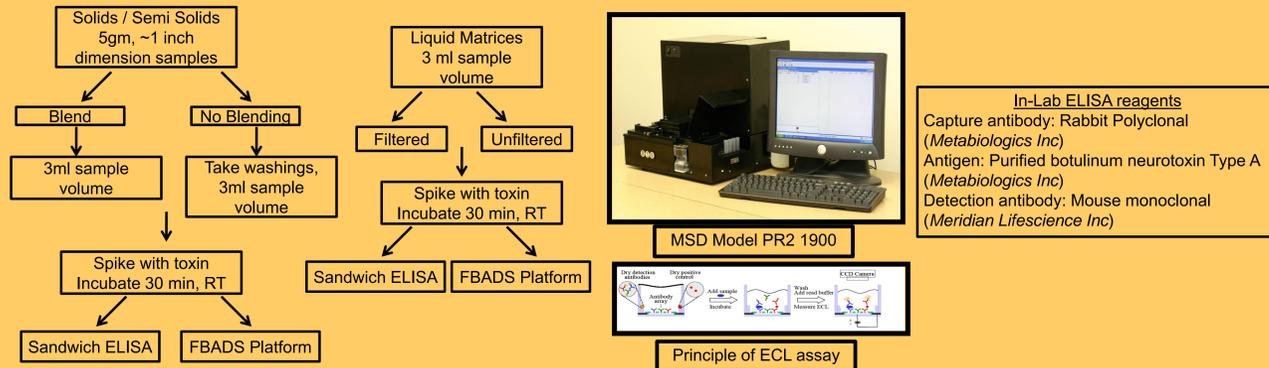
1. INTRODUCTION

Botulinum is a potent neurotoxin with lethal doses at sub nanomolar range making it a significant biothreat agent. There are seven serotypes of botulinum toxin - types A, B and E are commonly associated with illness in humans¹. Botulism is characterized by flaccid muscle paralysis. The lethal dose for mice is 0.3 ng/kg and for humans is thought to be 0.2-2.0 µg/kg². Since there is no known effective chemical antidote, the only therapeutic option is early detection and accurate determination of serotype so that the appropriate antitoxin can be administered. Similarly, preventive measures for detection of deliberate toxin food contamination include rapid detection and serotyping.

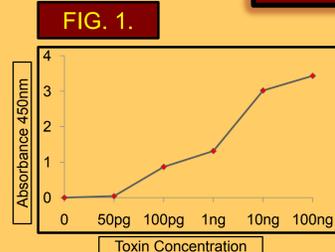
The current accepted diagnostic method for detection of botulinum toxin in foods is the mouse bioassay, which laborious, expensive, and lacks specificity. Toxin serotype specific in vitro detection is desperately needed to provide improved alternatives to animal testing and faster implementation of control programs in cases of outbreaks. In this study we evaluated the performance of a the MesoScale system (Gaithersburg, Maryland) that employs the principle of electrochemiluminescence detection of botulinum toxin under the Food Biological Agent Detection Sensor (FBADS) Program of the DHS. Their model PR2 1900 utilizes a kit for detection of toxin serotype A and is standardized for toxin detection in raw milk to up to 40pg/ml. Our goal is to use this FBADS system to test sample sets containing antigen at different concentrations across a variety of food matrices to include liquids, solids and semi-solids to verify the laboratory demonstration of limits of detection (LOD) and identification of toxin serotype. The detection and identification of the targeted antigens under realistic conditions in this assay will be compared to the diagnostic ability of standard lab-based colorimetric ELISA.

2. EXPERIMENTAL APPROACH

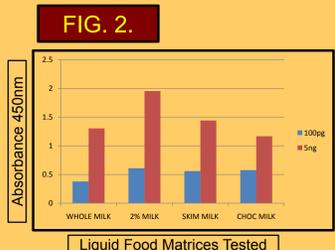
- Liquid, semisolid and solid food samples like milk, juices, vegetables, meat, cheese etc procured from local grocery stores were spiked with botulinum neurotoxin type A in varying concentrations ranging from 10-pg/ml to 10-ng/ml.
- Each concentration of the spiked food matrix will be tested in triplicate along with a no-toxin control on the FBADS platform as well as by a in-lab colorimetric sandwich ELISA
- Sensitivity of the ECL assay and ELISA will be evaluated and compared to define limits of detection



3. PRELIMINARY RESULTS (In-lab ELISA)

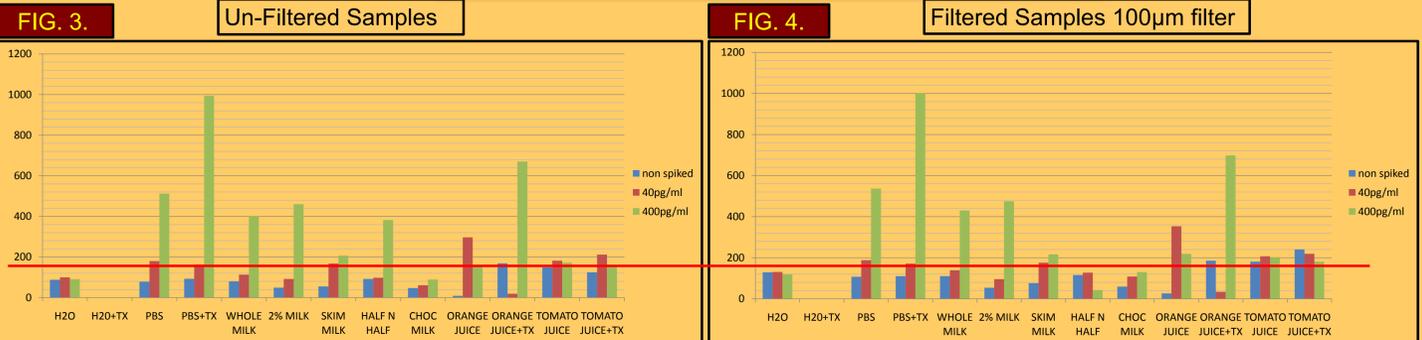


- The graph presented is generated using a colorimetric sandwich ELISA that is presently being standardized in the lab.
- Preliminary assays have demonstrated detection of at least 100-pg/ml of toxin as compared to 60-pg/ml detection sensitivity reported in literature.



- ELISA results of 100-pg/ml and 5-ng/ml botulinum toxin type A complex in some liquid food samples:-**
- The samples were unfiltered, spiked with neurotoxin and incubated for 30 minutes at room temperature.
 - Absorbance of negative controls was subtracted from the positive test samples
 - Minimum detected concentration in liquid matrices was 5ng/ml for these four samples.
- Currently we are extending the assay to include more food matrices and standardize the toxin extraction procedure to maximize the detection limit.

4. PERFORMANCE OF THE FBADS PLATFORM



MSD Model PR2 1900 ECL assay on liquid food matrices using the botulinum type A detection array plate

- The kit has been standardized by the manufacturers using raw milk: sample and sensitivity is described at detecting 40-pg/ml which corresponds to 160 ECL units
- In our study we tested for toxin detection in water, phosphate buffer, whole milk, 2% milk, skim milk, half & half, chocolate milk, orange juice and tomato juice (with and without addition of Triton-X)
- Using the cut off specifications of the manufacturers the results show-
 - ❖ **Unfiltered samples:-**
 - @ 400-pg/ml is detectable in all liquid matrices tested except in water and chocolate milk.
 - @ 40-pg/ml is detectable only in PBS and skim milk.
 - ❖ **Filtered samples:-**
 - @ 400-pg/ml is detectable in all liquid matrices except water, half & half and chocolate milk
 - @40-pg/ml is detectable only in PBS and skim milk.

5. CONCLUSIONS

- ❖ **MERITS:-**
 - Automated system
 - User friendly,
 - Simple software interface for viewing results
 - Runs 24 samples at one time, with 20 minutes time to result and 5 minutes between results.
- ❖ **LIMITATIONS:-**
 - Kit standardized for raw milk and has signal: noise interference with liquid matrices that are colored or have thicker consistency or non-neutral pH.
 - Instrument is not designed to deal with solid/semi solid matrices and require extensive sample preparation prior to testing.

6. ONGOING WORK

- Evaluate sensitivity & reproducibility of the ECL assay on liquid matrices.
- Evaluate performance on solid/semi solid food matrices, optimize sample preparation protocol
- Define limits of detection.

7. ACKNOWLEDGEMENTS / REFERENCES

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