BioSentinel

University Research Park
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25 June 2018

Dr. Warren Casey
NICEATM

Re: # Request for Data and Information on Technologies Used to Detect and Measure Botulinum Neurotoxin

Dear Dr. Casey,

BioSentinel Inc. (Madison, WI) specializes in the detection of botulinum neurotoxin (BoNT) with a focus on the development and commercialization of highly sensitive assays and methods compatible with a wide range of samples and matrices. Founded in 2007 and privately held, BioSentinel's customers and clients include multi-national and small pharmaceutical companies, non-profit and government food testing laboratories, US Department of Defense laboratories, and several other US and foreign government laboratories. We have successfully developed and transferred methods for relative potency testing of drug products and substances, high-throughput screening for BoNT inhibitors, and food testing. In addition, we have worked on or are currently working on collaborative projects for the potency testing of new BoNT-related medicines and the diagnosis of BoNT in wildlife samples. Because of our specialization and in-house expertise, we understand the need to develop products, methods, and services that are capable of detecting picogram quantities of BoNT in a wide-range of sample types.

A summary of our products and current services are listed below.

Products:

BoTest® BoNT Detections Kits

Description: These flexible assays provide real-time detection of BoNT proteolytic activity in an optimized reaction buffer. Intended uses include quantification and characterization of BoNT pharmaceutical preparations, drug discovery, high-throughput screening applications, and real-time detection applications.

Offerings:
A1004 BoTest A/E BoNT Detection Kit, 200 assays, for BoNT serotypes A and E
A1009 BoTest B/D/F/G BoNT Detection Kit, 200 assays, for BoNT serotypes B, D, F, and G
A1039 BoTest KO Non-specific Protease Detection Kit, 200 assays, a non-specific protease assay kit. The BoTest KO Kit can be used to control for false positives or to optimize assay conditions for samples that contain non-specific protease activity.

Reference: $
Ruge, D.R., et al., *Detection of six serotypes of botulinum neurotoxin using fluorogenic reporters.* Anal Biochem, 2011. **411**(2): p. 200-9. $

BoTest Matrix Botulinum Neurotoxin Detection Kits

Description: These serotype-specific assays are robust and sensitive tools for the detection and quantification of BoNT proteolytic activity contained in complex matrices

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such as pharmaceutical preparations, food samples, blood, and serum. These kits use magnetic bead-based immunological separation of BoNT from interfering substances found in complex matrices.

Offerings:
A1015 BoTest Matrix A BoNT Detection Kit, 200 assays, for BoNT serotype A
A1023 BoTest Matrix B BoNT Detection Kit, 200 assays, for BoNT serotype B
A1019 BoTest Matrix E BoNT Detection Kit, 200 assays, for BoNT serotype E
A1026 BoTest Matrix F BoNT Detection Kit, 200 assays, for BoNT serotype F

References:
1. $ Piazza, T.M., et al., *In vitro detection and quantification of botulinum neurotoxin type e activity in avian blood.* Appl Environ Microbiol, 2011. **77**(21): p. 7815-22.
2. $ Dunning, F.M., et al., *Detection of botulinum neurotoxin serotype a, B, and f proteolytic activity in complex matrices with picomolar to femtomolar sensitivity.* Appl Environ Microbiol, 2012. **78**(21): p. 7687-97.
3. Dunning, F.M., et al., *Isolation and quantification of botulinum neurotoxin from complex matrices using the BoTest matrix assays.* J Vis Exp, 2014(85).

BoLISA® Botulinum Neurotoxin Sandwich ELISAs

Description: These kits are used to detect and quantify total BoNT mass from complex matrices using a traditional sandwich ELISA approach. Each kit is serotype specific.

Offerings: $
A1029 BoLISA A BoNT Sandwich ELISA Detection Kit, 100 assays, uses an anti-BoNT/A capture antibody and a biotinylated anti-BoNT/A detection antibody. $
A1045 BoLISA B BoNT Sandwich ELISA Detection Kit, 100 assays, uses an anti-BoNT/B capture antibody and a biotinylated anti-BoNT/B detection antibody. $
A1042 BoLISA C BoNT Sandwich ELISA Detection Kit, 100 assays, uses an anti-BoNT/C capture antibody and a biotinylated anti-BoNT/C detection antibody. $
A1034 BoLISA E BoNT Sandwich ELISA Detection Kit, 100 assays, uses an anti-BoNT/E capture antibody and a biotinylated anti-BoNT/E detection antibody. $

BoLISA kits for serotypes B4 (subtype specific) and F are in development.

BoCell® Cell-based Assays (CBAs)

Description: These live-cell assays are intended for the relative potency testing of drug product or other BoNT-containing substances as well as for drug discovery. The CBA uses an engineered cell line that expresses a fluorescent reporter that responds to BoNT proteolytic activity after the toxin enters the cell. The BoCell CBAs can replace animal methods, where the ability to assay BoNT's cell recognition, translocation, and proteolytic activities is necessary.

Offerings: $
BoCell A/E CBA, detects BoNT serotypes A and E with sub-picomolar sensitivity. $
BoCell B CBA, for the detection of BoNT serotype B is in development. $

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Services:

Method and protocol development: BioSentinel has developed over 20 methods and protocols for clients who manufacture BoNT-based drug products and substances. The methods primarily include relative potency assays with drug product-specific sample preparations but also encompass stability protocols, cleaning and inactivation protocols, and qualification protocols. Most of these methods and protocols have been transferred to the client's laboratories or their contract laboratory. Several of the methods have been validated under GxP conditions.

BioSentinel has also developed methods for high-throughput screening for BoNT inhibitors, food sample testing, and testing for BoNT in wildlife samples.

Assay and method training and transfer: BioSentinel has transferred methods and/or trained operators in laboratories in four different countries.

Non-GxP sample testing: BioSentinel has tested hundreds of drug product and substance samples for clients with early stage drug programs. BioSentinel has also tested food and wildlife samples as part of collaborative projects.

Automation: BioSentinel has successfully developed automated and semi-automated methods for the detection of BoNT using our detection technologies. These methods reduce assay transfer time and reduce operator-to-operator variability.

Additional information can be found on our website (www.biosentinelpharma.com). BioSentinel has substantial data around many BoNT-related applications and, in many cases, the data can be shared upon request.

Sincerely,

Ward Tucker, Ph.D. $
Director of Research and Development $