ICCVAM/NICEATM/ECVAM Scientific Workshop on Alternative Methods to Refine, Reduce, and Replace the Mouse LD_{50} Assay for Botulinum Toxin Testing (November 13-14, 2006)

Poster Session Abstracts

Page Number/ Poster Board Number	Author List	Abstract Title
1	De Medici D, Fenicia L, Anniballi F, Delibato E, Aureli P	Polymerase Chain Reaction (PCR) as Alternative Method to Mouse Bioassay for Typing of Botulinum Neurotoxin-Producing Clostridia
2	Fenicia L, Anniballi F, Bolle P, Evandri MG, Martinoli L, Aureli P	Detection of Botulinum Neurotoxins Using *Daphnia magna* Toxicity Test
3	France R, McLaren J, Cox H, Banks L, Quirk R, Shakesheff K, Thompson D, Panjwani N, Shipley S, Pickett A	A New *Ex Vivo* Assay for Determining the Potency of Botulinum Type A Toxin-Hemagglutinin Complex (Dysport®): The Intercostal Neuromuscular Junction (NMJ) Assay
4	France R, McLaren J, Cox H, Banks L, Quirk R, Shakesheff K, Thompson D, Panjwani N, Shipley S, Pickett A	Validation of *Ex Vivo* Assays for the Potency Determination of Botulinum Toxins: The Impact of Experimental Variables in the Intercostal NMJ Assay
5	Gross GW	Quantification of BoNT-A Activity Suppressionin Neuronal Networks Growing on Microelectrode Arrays *In Vitro*
6	Huber A, Shakesheff KM, Pickett A	Rat Rib Cage-Derived Nerve-Muscle Preparations Provide a Reproducible Tissue Source for Use in Alternative *In Vitro* Potency Testing of Botulinum Toxin Preparations
7	Singh BR, Lindo P, Cai S	Endo-ELISA Bioassay of Live Botulinum Neurotoxins
8	Stanker LH, Merrill P	Development of High Affinity Monoclonal Antibodies Specific for Botulinum Neurotoxin Type A and a Sensitive Immunoassay with Detection Near that of the Mouse Bioassay
9	Torii Y, Takahashi M, Ishida S, Sakamoto T, Harakawa T, Ginnaga A, Kozaki S, Kaji R	Quantification of the Activity of Causing Flaccid Paralysis of Botulinum Neurotoxin by Measuring the Compound Muscle Action Potential (CMAP)
10	Xu J, Hang J, Lee JH, Zhu P, Amstutz P, Tang CM, Shelton D, Poli M, Rivera V, Shone CC	Assays to Detect Active Botulinum Neurotoxin

[This Page Intentionally Left Blank]

Polymerase Chain Reaction (PCR) as alternative method to mouse bioassay for typing of botulinum neurotoxin-producing clostridia.

De Medici D.*, Fenicia L., Anniballi F., Delibato E., and Aureli P.
National Reference Centre for Botulism, National Centre for Food Quality and Risk Assessment, Istituto Superiore di Sanità, Roma, ITALY

Botulism is a severe neuroparaliytic disease characterized by flaccid paralysis. Seven antigenically distinct toxin types (A, B, C_1, D, E, F and G) of the botulinal neurotoxins (BoNTs) have been identified [1]. Although BoNTs are classically produced by *Clostridium botulinum*, since 1979 other BoNT-producing species have been isolated. In particular, *Clostridium baratii*, which produces BoNT type F, has been isolated in the United States and in Hungary *Clostridium butyricum*, which produces BoNT type E, has been isolated in Italy, in China and in India. [2, 3]. Since different species of Clostridia have the ability to produce BoNTs, conventional isolation and identification of BoNTs-producing clostridia cannot rely solely on the basis of biochemical characteristics.
Currently, microbiological methods take into consideration only *C. botulinum* species, and the identification procedure included the confirmation and typing of the BoNTs production of the strain by mouse bioassay [4]. This technique is highly sensitive and specific, but costly, time-consuming, laborious, raises ethical concern due to the use of experimental animals, and does not take in consideration other BoNTs-producing clostridia. Moreover, efforts have been made to develop alternative methods to animal testing, as international legislation recommends (Directive 86/609/EEC).
The International Organization for Standardisation (ISO) has underlined the need to harmonize and standardize PCR-based methods to detect BoNTs-producing clostridia (Resolution 259 taken during the 24th Meeting of ISO TC34 SC9 – Warsaw – June 2005). The development of molecular biological methods based on the detection of BoNTs genes would be ideal. Different PCR methods have been described for detection of BoNTs-producing clostridia in food and clinical samples, and results obtained using PCR assays to detect neurotoxin gene fragments show a very high level of agreement with those from the mouse bioassay [5]. In our experience, where the typing of BoNTs-producing clostridia has been performed for two years using multiplex PCR and the confirmation of the production of the toxins using mouse bioassay halved the use of the animals in our laboratory. During this period we have analyzed about 400 samples (clinical and food) using a modified multiplex conventional PCR method and the all the results obtained were correctly confirmed by mouse bioassay.

References

1. Sobel J. 2005. Botulism. Clin Infect Dis; 41:1167-73.
2. Fenicia L., Franciosa G., Pourshaban M., and Aureli P.1999. Intestinal toxemia botulism in two young people, caused by *Clostridium butyricum* type E. Clin Infect Dis; 29(6):1381-7.
3. Anniballi F. Fenicia L., Franciosa G., and Aureli P. 2002. Influence of pH an temperature on the growth and toxin production by neurotoxigenic strains of *Clostridium butyricum* type E. J Food Prot; 65(8):1267-70.
4. CDC.1998. Botulism in United States, 1899-1966. Handbook for Epidemiologists, Clinical and Laboratory workers.
5. Akbulut D., Grant K.A., McLauchlin J. 2004. Development and application of Real-Time PCR assays to detect fragments of the *Clostridium botulinum* types A, B, and E neurotoxin genes for investigation of human foodborne and infant botulism. Foodborne Pathog Dis; 1(4): 247-57.

Detection of botulinum neurotoxins using *Daphnia magna* toxicity test

Fenicia L[1*]., Anniballi F[1]., Bolle P.,[2] Evandri M.G.[2], Martinoli L.[2], and Aureli P.[1]
[1]National Reference Centre for Botulism, National Centre for Food Quality and Risk Assessment, Istituto Superiore di Sanità, Roma, ITALY
[2]Department of Human Physiology and Pharmacology, University "La Sapienza", Roma, ITALY

Botulism is a rare disease naturally occurring or caused by accidental or intentional exposure to botulinum toxins (BoNTs).
BoNTs are a group of seven (A-G) antigenically distinct metalloproteinase classically produced by *Clostridium botulinum*, and by rare strains of *Clostridium baratii* and *Clostridium butyricum* [1]. All the toxins exert their action on the cholinergic system at the presynaptic motor-neuron terminal by blocking acetylcholine transmission across the neuromuscular junction, causing neuromuscular blockade, resulting in flaccid paralysis.
Since the 1970s, the therapeutic value of the paralytic potential of BoNTs for the treatment of muscular contraction was recognized and BoNT type A and B are now used in over 50 therapeutic and cosmetic preparations.
At present, the currently used method to detect and quantify the BoNTs is the Mouse Test that is complex, very expensive and entails the use of laboratory animals with ethical issues resulting.
The aim of this study was to assess whether *Daphnia magna* was a sensitive organism to detect BoNTs. The microcrustacean *Daphnia magna* Straus, besides being commonly used in standard toxicity tests as reference species, was reported to have a cholinergic system and contains cholinoreceptors identical (with respect to pharmacodynamic parameters) to those in human and animal organisms [2].
In this preliminary approach the *Daphnia magna* acute toxicity test was used. The experiments were conducted using cultures of *C. botulinum* type A, B and E, and were performed in accordance to the ISO Standard 6341 protocol [3] with minor modifications. In each test session, three replicates of treatment concentrations, including reference blank, and four replicates of control group, were applied. For each replicate, four *daphnids* less than 24-h old at the beginning of the test and coming from a third brood, were used.
Results showed that 300, 20, and 80 mouse MLD/ml of type A, B and E BoNTs respectively, gave a 100 % mortality at 48 h in *Daphnia magna* (reference blank group 0 % mortality). Specificity of the toxic effect was asserted exposing *daphnids* in medium containing botulinum culture and polyvalent antitoxin.
These results demonstrate that *Daphnia magna* is sensitive to BoNTs although less than the mouse. Further investigations using chronic toxicity test with end-points different from lethality (e.g. motility), could increase the test sensitivity.

References

1. Hatheway C.L. 1990. Toxigenic clostridia. Clin Microbiol Rew; 3(1):66-98
2. Podosinovikova NP, Kosmachev AB, Tonkopii VD, Zagrebin AO, Evdokimova EA, Malov AM, Petrov VV, Dolgo-Saburov VB. Daphnia magna for studies of cholinergic preparations. Eksp Klin Farmakol. 2002 Jan-Feb;65(1):73-4
3. ISO 6341. 1989. Water quality – determination of the inhibition of the motility of *Daphnia magna* Straus (Cladocera, Crustacea). International Organization for Standardization, Geneve, Switzerland.

A New Ex-Vivo Assay for Determining the Potency of Botulinum Type A Toxin-Hemagglutinin Complex (Dysport®): The Intercostal Neuromuscular Junction (NMJ) Assay.

Richard France[1], Jane McLaren[1], Helen Cox[1], Lisa Banks[1], Robin Quirk[1], Kevin Shakesheff[1], David Thompson[2], Naveed Panjwani[3], Sarah Shipley[3], Andy Pickett[3].

1. RegenTec Ltd, BioCity, Pennyfoot Street, Nottingham, NG1 1GF, UK.
2. David Thompson Applied Statistics, Unit 1, Abbey Enterprise Centre, Premier Way, Romsey, SO51 9AQ, UK.
3. Ipsen Biopharm Ltd, Ash Road North, Wrexham, LL13 9UF, UK.

Dysport® is a highly purified botulinum type A toxin complex used in a wide variety of indications within neurology, rehabilitation medicine and dermatology since 1990. The accurate determination of potency in clinical application of botulinum toxins such as Dysport® is critical to ensure clinical efficacy and safety, and is currently achieved using a lethal dose (LD_{50}) assay in mice. The increasing desirability of alternative methods for potency testing of botulinum toxin therapeutics is well established. As one such alternative, we have developed the *ex-vivo* Intercostal NMJ assay, which uses substantially fewer animals and does not involve the exposure of live animals to toxin. The assay records the decay of force from isolated and innervated intercostal muscle tissue sections stimulated electrically, and thus combines the important mechanisms of receptor binding, translocation and enzymatic action of the toxin molecule. Here we present data from a recent pre-validation study undertaken to determine the performance of the assay (including accuracy, precision and the replicate numbers required for routine use). Application of toxin to intercostal muscle results in the reduction of tissue lifetime in a dose dependant manner over a 0-60 Ipsen LD_{50} Unit dose range. Statistical analysis has been used to describe the relationship between applied dose and tissue lifetime. This regression model allows the prediction of toxin potency from the measured area under force decay curves. We show initial data demonstrating the assay provides accurate and precise results when tested with samples of well characterised reference batch material. Under the current methodology and within acceptable limits of confidence, potency determination of an individual batch of Dysport® requires a minimum of 60 tissue section replicates, currently achieved using a total of 12 animals.

Ethical and Legal Statement: All animal work was conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

Validation of Ex-Vivo Assays for the Potency Determination of Botulinum Toxins: The Impact of Experimental Variables in the Intercostal NMJ Assay.

Richard France[1], Jane McLaren[1], Helen Cox[1], Lisa Banks[1], Robin Quirk[1], Kevin Shakesheff[1], David Thompson[2], Naveed Panjwani[3], Sarah Shipley[3], Andy Pickett[3].

1. RegenTec Ltd, BioCity, Pennyfoot Street, Nottingham, NG1 1GF, UK.
2. David Thompson Applied Statistics, Unit 1, Abbey Enterprise Centre, Premier Way, Romsey, SO51 9AQ, UK.
3. Ipsen Biopharm Ltd, Ash Road North, Wrexham, LL13 9UF, UK.

Dysport® is a highly purified botulinum type A toxin complex used in a wide variety of indications within neurology, rehabilitation medicine and dermatology since 1990. The accurate determination of potency in clinical application of botulinum toxins such as Dysport® is critical to ensure clinical efficacy and safety, and is currently achieved using a lethal dose (LD_{50}) assay in mice. The increasing desirability of alternative methods for potency testing of botulinum toxin therapeutics is well established. We have developed the *ex-vivo* Intercostal NMJ assay as one such alternative. The NMJ assay measures the decay in force generated in electrically stimulated intercostal muscle tissue sections in response to toxin, and incorporates the combined mechanisms of action (receptor binding, translocation and enzymatic action) of the toxin molecule. Other *ex-vivo* methods such as those using isolated phrenic nerve hemi-diaphragm have been used assess toxin action, but details of their validation state are unclear. The aims of this study were to examine the effect of various test variables on the output of the NMJ assay during a pre-validation study using Dysport®. Here we report the effects of three such variables: animal weight, the side of animal from which the tissue originates and the position of the tissue within the ribcage. Using analysis of variance, after correction for other factors these variables are observed to have small but significant influence on the force decay curves and the subsequent predicted potency. Understanding and measuring the effect of these and other variables in *ex-vivo* assays is critical for the analytical method development process and assay validation.

Ethical and Legal Statement: All animal work was conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

Quantification of BoNT-A Activity Suppressionin Neuronal Networks Growing on Microelectrode Arrays *in vitro*.

Guenter W. Gross,
Department of Biological Sciences and Center for Network Neuroscience, University of North Texas, Denton. TX.

Spontaneously active neuronal networks cultured on microelectrode arrays (MEAs) can provide quantitative, multifaceted data on cytotoxicity, functional neurotoxicity, and pharmacological efficacy [(1,2,3)], where functional neurotoxicity is defined as the loss of network action potential (AP) production in the absence of cytotoxicity. One pregnant mouse with ten embryos can seed over 1,000 MEAs if several regions of the central nervous system are used. Each network can support one BoNT assay, providing remarkable animal and tissue utilization efficiency.

The primary response of these systems to BoNT-A is a gradual but irreversible reduction in spontaneous activity. The minimum concentration yielding reliable responses was 2 ng/ml, (13 picoMolar, using 150kD as the BoNT-A MW). Above this level, network responses were assessed at 100 ng/ml (n= 9), 50 ng/ml (n=11), 25 ng/ml (n=9), and 5 ng/ml (n=7). Delay times required to reach 50% and 90% activity reductions were concentration-dependent and could be approximated by power functions. At 100 ng/ml, these values were 2.8 h and 3.1 h, respectively, and reached 5 and 9 h at 10 ng/ml. Antisera provided by JVAP in 2001 (stored at -80°C) were used in 15 experiments. Sera additions ranging from 0.5 to 5% and high concentrations of BoNT (50-250 ng/ml) were used to ascertain protection. Of these, nine serum samples (selected at random) provided protection over long monitoring periods (up to 50 hours).

To perform such tasks rapidly and efficiently, it is desirable to use multinetwork platforms for parallel recording using robotic maintenance. Such platforms have reached the "proof of concept" stage under a Texas Advanced Technology Program grant, but require further development in the domains of automated data analysis and effective user interface programs. A 16-network system using the industry-standard 24 well plate is in the design stage.

References

(1) Gross, G.W and Gopal, K.V. (2006) Emerging histiotypic properties of cultured neuronal networks. In: M. Taketani and M. Baudry (eds) Advances in Network Electrophysiology using Multi-Electrode Arrays. Springer, pp 193-214.
(2) Xia, Y. and Gross, G.W. (2003) Histiotypic electrophysiological responses of cultured neuronal networks to ethanol. Alcohol 30: 167-174.
(3) Keefer, E.W., Gramowski, A., and Gross, G.W. (2001) NMDA receptor dependent periodic oscillations in cultured spinal cord networks. J. Neurophysiol. 86: 3030-3042.

Approved by the institutional animal care and use committee of the University of North Texas in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the Institute of Laboratory Animal Resources.

Rat rib cage-derived nerve-muscle preparations provide a reproducible tissue source for use in alternative *in vitro* potency testing of botulinum toxin preparations

Alexander Huber[1]
Kevin M Shakesheff[1,2]
Andy Pickett[3]

[1]Tissue Engineering Group, School of Pharmacy, University of Nottingham, UK
[2]RegenTec Ltd., Nottingham, UK
[3]Ipsen Biopharm Ltd, Ash Road North, Wrexham, LL13 9UF, UK.

Abstract

Since their introduction in the early 1980s, therapeutic Botulinum neurotoxin type A preparations have found an increasing number of clinical uses in a large variety of neuromuscular disorders and dermatological conditions. These treatments exploit the ability of the toxin to prevent neurotransmitter release from mainly cholinergic motor neurons, thereby resulting in a weakened contractile response. The use of therapeutic preparations of accurate potency in the clinical environment is crucial for correct patient treatment.
Currently, the potency of a preparation is determined by using the LD_{50} assay. However, operational and ethical concerns associated with this assay have prompted the development of alternative assays that could potentially become replacements. Several different *ex vivo* muscle-nerve preparations have been used to study botulinum intoxification, including isolated muscle preparations of the extensor digitorum longus (EDL), and phrenic nerve-hemidiaphram. These are models on which potential alternative assays may be based, however, incomplete evaluations of their quantitative performance and robustness have hindered their use as replacement test methods. Recently, Ipsen (Wrexham, UK) in collaboration with RegenTec (Nottingham, UK) have developed the use of isolated rat intercostal rib sections in an alternative *ex vivo* neuromuscular junction assay (the "Intercostal NMJ Assay"). This alternative assay addresses ethical and operational constraints of a live animal assay. Here, we demonstrate that the six individual rat intercostal nerve-muscle preparations isolated from a single animal's rib cage provide a valuable source of highly similar preparations for an *ex vivo* neurotoxicity assay. A high degree of structural similarity was observed in terms of the anatomy of sections, as highlighted by the patterns of innervating neuromuscular junctions identified by acetylcholinesterase staining. Similarly, the physiological nature of the intercostal muscle sections was determined by their level of succinic dehydrogenase activity. Again, no significant differences were observed between sections. In summary, our results confirm that independent intercostal nerve-muscle preparations are anatomically and physiologically similar in nature supporting their use in an *ex vivo* alternative potency test such as the Intercostal NMJ Assay.

Ethical and Legal Statement: All animal work was conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

Endo-ELISA Bioassay of Live Botulinum Neurotoxins

Bal Ram Singh[1,2], Paul Lindo[1,2,3], and Shuowei Cai[1,2]
[1]Botulinum Research Center, [2]Department of Chemistry and Biochemistry, University of Massachusetts Dartmouth; and [3]BBTech, Inc., Dartmouth, MA 02747

We have developed antibodies against purified and complex forms of botulinum neurotoxins which can detect the toxin below 50 pg/ml concentration in a sandwich format, without any additional amplification. However, this approach only provides detection of the toxin without any distinction of denatured or live toxin state. To address this issue, we have designed an approach in which the toxin is captured on a microtiter plate by either monoclonal or polyclonal antibodies raised against the heavy chain of the type A botulinum neurotoxin (BoNT/A). The captured toxin is subsequently incubated with BoNT/A neuronal substrate, SNAP-25-GST fusion protein or His-tagged SNAP-25, and its cleavage is followed by subsequent determination of the cleaved product either by anti-SNAP-25 IgG directly or after capturing the product with anti-GST antibodies. In both cases the results suggested that antibody captured BoNT/A was functional and its endopeptidase activity was monitored in the enzyme linked immunosorbent assay (referred to as Endo-ELISA bioassay).

The results showed that BoNT/A bound to anti-heavy chain monoclonal/polyclonal antibody cleaved the SNAP-25 in a time dependent manner, and significant cleavage occurred within the first 20 min. After 60 min incubation about 70% SNAP-25 was cleaved. This set of experiments establish the following points relevant to the use of this approach for designing non-animal based bioassay of botulinum neurotoxins. Anti-BoNT/A heavy chain IgG was able to capture the toxin on the microplate wells, the captured BoNT/A was enzymatically active, and the enzyme activity was strong enough to show significant SNAP-25 cleavage within the first 20 min of reaction.

The Endo-ELISA bioassay was found to be effective for purified as well as native BoNT/A complex, and it worked in a variety of matrices, such as milk, wine, and honey.

Development of High Affinity Monoclonal Antibodies Specific for Botulinum Neurotoxin Type A and a Sensitive Immunoassay with Detection Near that of the Mouse Bioassay

Larry H Stanker and Paul Merrill
USDA - Agriculture Research Service
800 Buchanan St.
Albany, CA 94710

Clostridium botulinum neurotoxins (BoNT) cause severe and potentially fatal neuroparalytic disease and are considered the most toxic biological agents known. BoNT is synthesized as a single 150 kDa precursor protein, which is cleaved to form two subunit polypeptides, linked by a single disulfide bond. The 'gold standard' for BoNT detection is the mouse bioassay. While the assay it is time consuming (up to 4 days) and lacks specificity, it has a limit of detection in the low picogram range. Most BoNT immunoassays reported appear to have much less sensitive than the mouse bioassay. In this study we describe the development of high affinity monoclonal antibodies (Mab). These are IgG_1 and IgG2b subclass MAb's with kappa light chains. They specifically bind BoNT serotype A and have measured Kd values in the low pM range. Western blot analysis demonstrated that four of the Mabs specifically bind the 100 kDa heavy-chain subunit, in while one of the antibodies specifically binds the 50Kda light-chain. Using a simple sandwich immunoassay format with a heavy-chain specific Mab for capture, a directly labeled anti light-chain Mab for detection and a luminescent substrate, detection of BoNT type A in the low picogram range was observed. Further characterization of these MAb and their application to rapid immunoassay formats will be discussed.

QUANTIFICATION OF THE ACTIVITY CAUSING FLACCID PARALYSIS OF BOTULINUM NEUROTOXIN BY MEASURING THE COMPOUND MUSCLE ACTION POTENTIAL (CMAP)

Yasushi Torii[1], Motohide Takahashi[2], Setsuji Ishida[2], Takashi Sakamoto[3], Tetsuhiro Harakawa[1], Akihiro Ginnaga[1], Shunji Kozaki[4], Ryuji Kaji[3] ([1]Kaketsuken, Japan, [2]National Institute of Infectious Diseases, Japan, [3]University of Tokushima, Japan and [4]Osaka Prefecture University, Japan)

Purpose

Quantitative determination of botulinum toxin usually depends on the LD50 method after mouse intraperitoneal injection. The index of this method is the lethal activity of the toxin, and the method requires a number of mice. As the method of direct determination of flaccid paralysis after administration of the toxin, the method for testing quality of toxin by determining compound muscle action potential (CMAP) was examined.

Materials and methods

Type A botulinum toxin (S or LL) solution was diluted serially so as to obtain 9 graded doses. Each dose in 0.1 ml was injected into left hind gastrocnemius muscle of a group of 5 anesthetized mice (SD strain). By use of stimulatory and recording electrodes inserted in the gastrocnemius muscle near the lumbar vertebra, the maximum CMAP amplitude of the injected muscle was determined by electromyography.

Results and discussions

CMAP amplitude was on the decrease toward the fourth day after administration of the toxin, and then recovered gradually. Statistical analysis was made on the data of the 1st , the 2nd, the 4th, the 7th and the 14th day of administration. For example, dose response was found on each dose, and a linear relation was observed between 0.01 and 30 mouse ip LD50/body on the 1st day (and between 0.01 and 1.0 mouse ip LD50/body on the 4th day), indicating the possibility of the use of CMAP method for quantitation of botulinum toxin.

All studies were carried out in accordance with the guidelines of the Japanese Society of Pharmacology on experimental animals and with approval of the Animal Ethics Committee of our institute.

Assays to Detect Active Botulinum Neurotoxin

Jing Xu, Jun Hang, Jia-Hai Lee, Peixuan Zhu, Pete Amstutz, and Cha-Mei Tang
Creatv MicroTech, Inc., Potomac, MD 20854

Daniel Shelton
US Department of Agriculture – Agricultural Research Service, Beltsville, MD 20705

Mark Poli and Victor Rivera
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702

Clifford C. Shone
Health Protection Agency, Porton Down, Salisbury SP4 0JG Wiltshire, United Kingdom

Active Botulinum Neurotoxin (BoNT) serotype A cleaves SNAP-25 peptide at a specific location. Detection of the cleaved SNAP-25 provides information on the activity and serotype of the toxin. The assays are based on the use of antibody specific to SNAP-25 cleaved by BoNT serotype A developed by Dr. Shone of Health Protection Agency. A proof-of-principle enzymatic activity assay is demonstrated.

The detection platform is the Integrating Waveguide Biosensor, which uses capillary tube waveguides to detect low level fluorescence signals. In the test, BoNT samples are incubated with a proprietary form of the SNAP-25 substrate, and the enzymatic reaction product is detected by fluorescent immunoassay inside the capillary waveguide. Excitation light impinges on the waveguide at a 90º angle. The emitted fluorescence signal is gathered efficiently by the waveguide and exits at one end through a set of lenses and optical filters to an optical detector. Emission light from the entire waveguide is integrated, thus increasing the detected signal, while background noise is minimized by excitation at a 90º angle.

Preliminary data indicates a detection level of 500 pg/ml in a three hour test. Optimization of the assay is expected to improve the limit of detection in the future.