

**[0013]** U.S. Pat. No. 5,962,637 to Shone et al, uses synthetic peptide substrates in a fluorescence resonance energy transfer (FRET) based solid-phase microtitre based in vitro assay using antibodies that recognize only post-proteolytic cleavage sites.

**[0014]** The assays of Schmidt et al. and Shone et al. only work in vitro so they only measure the proteolytic activity of BoNTs. The various embodiments of the assay of this invention work in living systems and, therefore, can be used to measure additional steps of intoxication; most notably toxin entry into cells. For example, small molecules that prevented toxin entry into cells would register as a potential lead compound in a BACS antibody/cellular based compound screen but not in a screen that only measured proteolytic activity in vitro.

**[0015]** The assays of Schmidt et al. and Shone et al. could be used for compound screening and evaluation in vitro (biochemical assay system). The various assays disclosed as embodiments of this invention could also be used to evaluate and screen compounds in vitro but more importantly compounds could be evaluated in cellular models of intoxication which are more stringent models for drug development. BACS antibody/cellular evaluation would allow important issues such as compound toxicity, bioavailability and intracellular efficacy to be assessed during primary screens. These parameters are not measurable in cell free systems.

**[0016]** The assays of Schmidt et al. and Shone et al. could be used to measure the activity of batches of BoNTs in vitro for quality control purposes. However, these assays only measure the proteolytic activity of the light chain. The BACS antibody/cellular assays of the various embodiments of the present invention would provide a more stringent evaluation of BoNT samples (which are often produced in bulk for medical applications) as they allow additional properties of the toxin to be assessed. Toxin entry into cells is mediated entirely by the toxin's heavy chain. A cellular assay of the toxin's activity (such as disclosed in the present application) allows the heavy chain mediated entry activity to also be evaluated in addition to the proteolytic activity mediated by the toxin's light chain. In vitro assays such as those designed by Schmidt et al. and Shone et al. only measure a single property (the proteolytic activity) of the toxin, thus batches of toxin with defective heavy chains but functional light chains would register as acceptable by in vitro assays but not by more stringent BACS/cell based assays.

**[0017]** The BACS antibody based assays could also be used in other living systems to study the effects of BoNTs in vivo, such as, by way of nonlimiting example, measurement of SNAP-25 cleavage in laboratory animals or patients from biopsy (by immunofluorescence microscopy). These studies will help understand the pharmacokinetics of the toxin in whole animal systems and allow the neuron function of patients suffering from botulism to be evaluated. Neither of these applications are possible using currently available technology.

#### SUMMARY

**[0018]** Botulinum neurotoxins (BoNTs) are zinc-metalloproteases that cleave components of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein complex, inhibiting acetylcholine release into neuromuscular junctions, resulting in flaccid paralysis and eventual death. The potential for the malicious misuse of these toxins as bioweapons has created an urgent need to

develop effective therapeutic countermeasures. Robust cell-based assays will be essential for lead identification and the optimization of therapeutic candidates. The various embodiments of the present invention include novel BoNT serotype A (BoNT/A) cleavage-sensitive (BACS) antibodies that only interact with full-length SNAP-25 (synaptosomal-associated protein of 25 kDa), the molecular target of the BoNT/A serotype (FIG. 1). These antibodies exhibit high specificity for full-length SNAP-25, allowing the BoNT/A-mediated proteolysis of this protein to be measured in diverse assay formats, including several variations of ELISA (enzyme-linked immunosorbent assay) and multiple immunofluorescence methods. Assays built around the BACS antibodies have excellent sensitivity, excellent reproducibility, and are amenable to multi-well formats. Furthermore, the various embodiments of the invention also include novel methods for evaluating BoNT/A activity in cellular models of intoxication and high-throughput evaluation of experimental compounds.

**[0019]** One embodiment of the invention relates to an assay for botulinum toxin or tetanus toxin comprising the steps of:

**[0020]** (a) combining a test compound with a substrate and with antibody, wherein the substrate has a cleavage site for the toxin and when cleaved by toxin forms a product, and wherein the antibody binds to the substrate but not to the product; and

**[0021]** (b) testing for the presence of antibody bound to the substrate.

**[0022]** Preferably, in the practice of this invention, the substrate is a peptide or a protein which is cleaved by the toxin to generate new peptide products. The assay according to the invention may utilize an antibody that binds to the substrate peptide but not to the cleavage products and may comprise the steps of: (a) combining a test compound with the substrate peptide to form an assay mixture, wherein the substrate peptide is selected from intact peptides or fragments thereof selected from the group consisting of VAMP; a VAMP analog; a VAMP isoform; SNAP-25; a SNAP-25 analog; a SNAP-25 isoform; syntaxin; a syntaxin analog; and a syntaxin isoform; or a fragment thereof; (b) combining the assay mixture with the antibody, and (c) determining whether there has been formed any conjugate between the antibody and the substrate.

**[0023]** In one embodiment, the assay further comprises of an antibody adapted selectively to bind to a peptide selected from a group consisting of SEQ ID NOS: 1, 2 and 3.

**[0024]** The present invention also relates to a method of obtaining an antibody adapted selectively to bind to the scissile bond of a toxin's molecular target, enabling toxin-associated proteolysis to be measured in a variety of assay formats, the method comprising identifying at least one antigenic peptide that correspondent to the toxin's cleavage site in the molecular target, immunizing an animal against at least one said antigenic peptide, isolating antibodies that bind to said antigenic peptide(s) and recovering said antibody.

**[0025]** In one embodiment, the method of obtaining an antibody adapted selectively to bind to the scissile bond of a toxin's molecular target preferably comprises immunizing an animal with an antigen selected from a group consisting of: SEQ ID NOS 1, 2 and 3 and a carrier molecule, and isolating the antibody that binds to said antigen. Acceptable carrier molecules are well known in the art and include, by way of non-limiting example: Keyhole Limpet Hemocyanin, Bovine Serum Albumin and Ovalbumin.