Testing of Botulinum Toxin Type A, Altern. Lab. Anim. 34(3): 305-313 (2006). In recent years, several steps have been already taken to reduce and refine the mouse LD_{50} bioassay in order to standardize the protocol and produce more consistent data using fewer animals per assay.

[04] Thus, a simple, reliable, validated and governmental agency acceptable botulinum toxin activity assay that can evaluate the integrity of all the steps necessary in botulinum toxin uptake would be of significant value because such a non-animal based assay would alleviate the need for animal testing and all the disadvantages, costs and ethical concerns associated with this type of animal-based assay. The present specification provides novel compositions, cells, and methods for assaying the activity of a botulinum toxin A useful for various industries, such as, *e.g.*, the pharmaceutical and food industries, and provides related advantages as well. Such compositions, cells, and methods do not use live animals or tissues taken from live animals, but can evaluate all the steps necessary for neurotoxin action.

SUMMARY

[04a] In a first aspect, there is provided a method of quantifying botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample comprising BoNT/A or suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody comprising a heavy chain variable region comprising complementarity determining regions (CDRs) comprising the amino acid sequences of SEQ ID NOs: 95, 99, and 101 and a light chain variable region comprising CDRs comprising the amino acid sequences of SEQ ID NOs: 103, 108, and 113 that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$;

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 comprising the C-terminal amino acid sequence of SEQ ID NO: 38; and

e. quantifying the level of BoNT/A activity in the sample, wherein the higher the amount of the antibody-antigen complex detected the higher the level of BoNT/A activity in the sample.

[04b] In a second aspect, there is provided a method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody comprising a heavy chain variable region comprising complementarity determining regions (CDRs) comprising the amino acid sequences of SEQ ID NOs: 95, 99, and 101 and a light chain variable region comprising CDRs comprising the amino acid sequences of SEQ ID NOs: 103, 108, and 113 that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

[04c] In a third aspect, there is provided a method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a SiMa cell (DSMZ No. ACC 164) with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

[04d] In a fourth aspect, there is provided a method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody is produced against a fusion protein consisting of the peptide of SEQ ID NO: 38 adjoined to a carrier protein, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

[04e] In a fifth aspect, there is provided a method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody that specifically binds to the peptide of SEQ ID NO: 38, wherein said antibody specifically binds to an epitope of said fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38 with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

DETAILED DESCRIPTION OF THE DRAWINGS

[05] **FIG. 1** shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where Clostridial toxin binds to a Clostridial receptor complex and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing a toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including changes in the internal pH of the vesicle, formation of a channel pore comprising the H_N domain of Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the light chain and 4) enzymatic target modification, where the light chain of Clostridial toxin proteolytically cleaves its target SNARE substrates, such as, *e.g.*, SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[06] **FIG. 2** shows a comparison of BoNT/A uptake in four cell lines by Western blot analysis. FIG. 2A shows a graph of SNAP-25 cleavage product detected based ~~on~~ amount of BoNT/A used to treat the cell line. The data were analyzed in SigmaPlot using a 4 parameter logistic model and EC_{50} values were obtained for each cell line. Ranking of SNAP-25 cleavage product signals detected was: SiMa>> Neuro-2a>LA1-55n> PC12. FIG. 2B shows the signal-to-noise ratios of the raw signals at 300 pM vs. 0 pM and

What is claimed:

1. A composition comprising a carrier, a flexible linker and a SNAP-25 antigen, wherein the flexible linker comprises at least three amino acids and the SNAP-25 antigen comprises SEQ ID NO: 148.

2. The composition of Claim 2, wherein the flexible linker and the SNAP-25 antigen amino acid sequence is SEQ ID NO: 38.

3. An isolated α-SNAP-25 antibody,

   wherein the isolated α-SNAP-25 antibody binds an eptiope comprising a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product;

   wherein the α-SNAP-25 antibody has an association rate constant for an epitope not comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product of less than 1×10^{1} M^{-1} s^{-1}; and

   wherein the α-SNAP-25 antibody has an equilibrium disassociation constant for the epitope of less than 0.450 nM.

4. The isolated α-SNAP-25 antibody of Claim 3, wherein the epitope is SEQ ID NO: 32.

5. An isolated α-SNAP-25 antibody comprising,

   a. a heavy chain variable region comprising SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 80, or SEQ ID NO: 82; and

   b. a light chain variable region comprising SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, or SEQ ID NO: 92.

6. An isolated α-SNAP-25 antibody comprising,

   a. a heavy chain variable region comprising SEQ ID NO: 93, SEQ ID NO: 121, or SEQ ID NO: 100; and

   b. a light chain variable region comprising SEQ ID NO: 105, SEQ ID NO: 110, or SEQ ID NO: 115.

7. An isolated α-SNAP-25 antibody comprising at least a V_H CDR3 of SEQ ID NO: 100, a V_H CDR3 of SEQ ID NO: 101, or a V_H CDR3 of SEQ ID NO: 102.

8. A method of detecting BoNT/A activity, the method comprising the steps of:

   a. treating a cell from an established cell line with a sample comprising a BoNT/A, wherein the cell from an established cell line is susceptible to BoNT/A intoxication by about 500 pM or less of a BoNT/A;

   b. isolating from the treated cell a SNAP-25 component comprising a SNAP-25 cleavage product having a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond;

   c. contacting the SNAP-25 component with an α-SNAP-25 antibody linked to a solid phase support,

      wherein the α-SNAP-25 antibody binds an eptiope comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product, the α-SNAP-25 antibody has an association rate constant for an epitope from a SNAP-25 not comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product of less than 1×10^1 M^{-1} s^{-1}; and the α-SNAP-25 antibody has an equilibrium disassociation constant for the epitope of less than 0.450 nM;

   d. detecting the presence of an antibody-antigen complex comprising the α-SNAP-25 antibody and the SNAP-25 cleavage product having a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond;

   wherein detection by the antibody-antigen complex is indicative of BoNT/A activity.

9. The method of Claim 8, wherein the SNAP-25 cleavage product is SNAP-25_{197}.

10. The method of Claim 8, wherein the presence of an antibody-antigen complex is detected using a sandwich ELISA.

11. The method of Claim 8, wherein the method has a signal-to-noise ratio at the lower asymptote of at least 3:1 and a signal-to-noise ratio at the upper asymptote of at least 10:1.

12. The method of Claim 8, wherein the sample comprises at most 100 pM of a BoNT/A

13. The method of Claim 8, wherein the cell from an established cell line is susceptible to BoNT/A intoxication by about 100 pM or less of a BoNT/A.

14. The method of Claim 8, wherein the method is performed in a singleplex fashion or a multiplex fashion.

15. A method of determining BoNT/A immunoresistance in a mammal comprising the steps of:

a. adding a BoNT/A to a test sample obtained from a mammal being tested for the presence or absence of α-BoNT/A neutralizing antibodies;

b. treating a cell from an established cell line with the test sample, wherein the cell from an established cell line is susceptible to BoNT/A intoxication;

c. isolating from the treated cells a SNAP-25 component comprising a SNAP-25 cleavage product having a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond;

d. contacting the SNAP-25 component with an α-SNAP-25 antibody linked to a solid phase support, wherein the α-SNAP-25 antibody;

wherein the α-SNAP-25 antibody binds an epitope comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product, the α-SNAP-25 antibody has an association rate constant for an epitope from a SNAP-25 not comprising a carboxyl-terminus glutamine of the BoNT/A cleavage site scissile bond from a SNAP-25 cleavage product of less than 1×10^{1} M^{-1} s^{-1}; and the α-SNAP-25 antibody has an equilibrium disassociation constant for the epitope of less than 0.450 nM;

e. detecting the presence of an antibody-antigen complex comprising the α-SNAP-25 antibody and the SNAP-25 cleavage product having a carboxyl-terminus glutamine from the BoNT/A cleavage site scissile bond;

f. repeating steps b-e with a negative control sample instead of a test sample, the negative control sample comprising a BoNT/A and a serum known not to contain α-BoNT/A neutralizing antibodies; and

g. comparing the amount of antibody-antigen complex detected in step e to the amount of antibody-antigen complex detected in step f,

wherein detection of a lower amount of antibody-antigen complex detected in step e relative to the amount of antibody-antigen complex detected in step f is indicative of the presence of α-BoNT/A neutralizing antibodies.

What is claimed:

1. A method of quantifying botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

   a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample comprising BoNT/A or suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

   b. isolating polypeptides from the cell;

   c. contacting the polypeptides with a monoclonal antibody comprising a heavy chain variable region comprising complementarity determining regions (CDRs) comprising the amino acid sequences of SEQ ID NOs: 95, 99, and 101 and a light chain variable region comprising CDRs comprising the amino acid sequences of SEQ ID NOs: 103, 108, and 113 that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$;

   d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 comprising the C-terminal amino acid sequence of SEQ ID NO: 38; and

   e. quantifying the level of BoNT/A activity in the sample, wherein the higher the amount of the antibody-antigen complex detected the higher the level of BoNT/A activity in the sample.

2. The method of claim 1, wherein the presence of an antibody-antigen complex is detected using a sandwich ELISA.

3. The method of claim 1, wherein the method has a signal-to-noise ratio at the lower asymptote of at least 3:1 and a signal-to-noise ratio at the upper asymptote of at least 10:1.

4. The method of claim 1, wherein the sample comprises at most 100 pM of BoNT/A.

5. The method of claim 1, wherein the established cell line is susceptible to said BoNT/A intoxication at about 100 pmol BoNT/A per liter culture medium.

6. The method of claim 1, wherein the method is performed in a singleplex fashion.

7. The method of claim 1, wherein the method is performed in a multiplex fashion.

8. The method of claim 1, wherein said antibody is linked to a solid phase support.

9. The method of claim 1, wherein said established cell line is the SiMa cell line (DSMZ No. ACC 164).

10. The method of claim 1, wherein said established cell line is the Neuro-2a cell line (ATCC Catalog No. CCL-131™).

11. The method of claim 1, wherein said cell is differentiated.

12. A method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said

SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody comprising a heavy chain variable region comprising complementarity determining regions (CDRs) comprising the amino acid sequences of SEQ ID NOs: 95, 99, and 101 and a light chain variable region comprising CDRs comprising the amino acid sequences of SEQ ID NOs: 103, 108, and 113 that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

13. The method of claim 12, wherein said antibody is linked to a solid phase support.

14. The method of claim 12, wherein said established cell line is the SiMa cell line (DSMZ No. ACC 164).

15. The method of claim 12, wherein said established cell line is the Neuro-2a cell line (ATCC Catalog No. CCL-131™).

16. The method of claim 12, wherein said cell is differentiated.

17. The method of claim 16, wherein said cell is from an established cell line selected from SiMa cell line (DSMZ No. ACC 164), Neuro-2a cell line (ATCC Catalog No. CCL-

131™), N18 (ECACC No. 88112301), LA1-55n (ECACC No. 06041203), PC12 (ATCC Catalog No. CRL-1721™), and SH-SY5Y (ATCC Catalog No. CRL-2266™).

18. The method of claim 12, wherein said cell line is transfected with a nucleic acid molecule encoding an exogenous BoNT/A receptor selected from FGFR2 and FGFR3.

19. A method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a SiMa cell (DSMZ No. ACC 164) with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

20. The method of claim 19, wherein said cell is transfected with a nucleic acid encoding an exogenous SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A.

21. The method of claim 19, wherein said cell is differentiated.

22. The method of claim 19, wherein said cell is transfected with a nucleic acid molecule encoding an exogenous BoNT/A receptor selected from FGFR2 and FGFR3.

23. A method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody that specifically binds to a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein said antibody is produced against a fusion protein consisting of the peptide of SEQ ID NO: 38 adjoined to a carrier protein, wherein said antibody binds an epitope of said fragment comprising the carboxylated carboxyl-terminal glutamine with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.

24. The method of claim 23, wherein said carrier protein is selected from a keyhole limpet hemocyanin (KLH), ovalbumin (OVA), thyroglobulin (THY), bovine serum albumin (BSA), soybean trypsin inhibitor (STI), and multiple attachment peptide (MAP).

25. A method of detecting botulinum neurotoxin serotype A (BoNT/A) activity in a sample, the method comprising the steps of:

a. contacting a cell from an established cell line expressing a SNAP-25 polypeptide consisting essentially of at least a portion of human SNAP-25 comprising SEQ ID NO: 5 cleavable by BoNT/A with a sample suspected of comprising BoNT/A, wherein the established cell line is susceptible to BoNT/A intoxication at about or less than 500 pmol BoNT/A per liter culture medium, as indicated by the enzymatic cleavage of said SNAP-25 polypeptide by BoNT/A to yield a fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38;

b. isolating polypeptides from the cell;

c. contacting the polypeptides with a monoclonal antibody that specifically binds to the peptide of SEQ ID NO: 38, wherein said antibody specifically binds to an epitope of said fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid sequence of SEQ ID NO: 38 with an equilibrium disassociation constant of less than 0.450 nM and wherein said antibody has an association rate constant for an epitope of intact SNAP-25 polypeptide comprising SEQ ID NO: 5 of less than $1\times10^{1}\ M^{-1}\ s^{-1}$; and

d. detecting the presence of any antibody-antigen complex comprising the antibody and the fragment of said SNAP-25 polypeptide comprising the C-terminal amino acid

sequence of SEQ ID NO: 38, wherein the higher the amount of the antibody-antigen complex detected the higher the amount of BoNT/A in the sample.