

Dear Dr Casey,

I have retired from NIBSC last year and no longer work actively on this topic. However during my time at NIBSC various in vitro methods have been developed and applied during independent evaluation of one brand of Botulinum toxin A product used in UK/EU as a consistency indicating test. Animals have not been used for I think more than 15 years for this purpose. Much of that was discussed during the last NICEATM meeting in 2008. Since that time we have developed a series of different approaches all using the same targeted antibodies that we made against specific cleavage sites on SNAP25 (for BoNT/A & /E assays) and VAMP2 (BoNT/B assays) proteins. These assays, unlike the first generation methods, measure several key functions of the toxin. I think that titles and information provided within these publications are self explaining.

Assessment of ELISA as endpoint in neuronal cell-based assay for BoNT detection using hiPSC derived neurons. Pellett S, Tepp WH, Johnson EA, Sesardic D. J Pharmacol Toxicol Methods. 2017 Nov;88(Pt 1):1-6. doi: 10.1016/j.vasch.2017.04.013. Epub 2017 Apr 29. PMID: 28465161

<https://reader.elsevier.com/reader/sd/A85681C84088700BF1B384FFDB3DD4AA7ACED99700715693704F95ED9D07B004EA426F302845C7312421D458C77D7FE7>

A Cell Line for Detection of Botulinum Neurotoxin Type B. Rust A, Doran C, Hart R, Binz T, Stickings P, Sesardic D, Peden AA, Davletov B. Front Pharmacol. 2017 Nov 9;8:796. doi: 10.3389/fphar.2017.00796. eCollection 2017.

PMID: 29170639

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5684488/pdf/fphar-08-00796.pdf>

SiMa Cells for a Serotype Specific and Sensitive Cell-Based Neutralization Test for Botulinum Toxin A and E. Bak N, Rajagopal S, Stickings P, Sesardic D. Toxins (Basel). 2017 Jul 20;9(7). pii: E230. doi: 10.3390/toxins9070230.

PMID: 28726719

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5535177/pdf/toxins-09-00230.pdf>

Immuno-detection of cleaved SNAP-25 from differentiated mouse embryonic stem cells provides a sensitive assay for determination of botulinum A toxin and antitoxin potency.

Yadirgi G, Stickings P, Rajagopal S, Liu Y, Sesardic D. J Immunol Methods. 2017

Dec;451:90-99. doi: 10.1016/j.jim.2017.09.007. Epub 2017 Sep 21.

PMID: 28943257

<https://reader.elsevier.com/reader/sd/EA3DDAC03A74EF3F31337D7B16A605CCA722F7D950F4BA51A3A741C9FA7608A047A1456C85F7A23590C324D64015EBBA>

A functional dual-coated (FDC) microtiter plate method to replace the botulinum toxin LD50 test. Liu YY, Rigsby P, Sesardic D, Marks JD, Jones RG. Anal Biochem. 2012 Jun

1;425(1):28-35. doi: 10.1016/j.ab.2012.02.038. Epub 2012 Mar 6.

PMID: 22406430

[https://ac.els-cdn.com/S0003269712001558/1-s2.0-S0003269712001558-main.pdf?\\_tid=020d03e0-0e3d-4bc2-b182-19b9c6f87962&acdnat=1529390536\\_aed70a5dc77a16d41ca48fe6ba975c53](https://ac.els-cdn.com/S0003269712001558/1-s2.0-S0003269712001558-main.pdf?_tid=020d03e0-0e3d-4bc2-b182-19b9c6f87962&acdnat=1529390536_aed70a5dc77a16d41ca48fe6ba975c53)

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