

Evolution of an assay for inhibitors of Botulinum neurotoxin A: from model system to high content. Gordon Ruthel<sup>1</sup>, Jon Nuss<sup>2</sup>, Krishna Kota<sup>3</sup>, and Sina Bavari. USAMRIID, Fort Detrick, MD 21702 and <sup>1</sup>Akimeka Technologies, <sup>2</sup>TRUE Foundation, <sup>3</sup>Perkin Elmer.

Botulinum neurotoxin (BoNT) is an exceptionally potent toxin that cleaves proteins of the SNARE complex, blocks release of acetylcholine into the neuromuscular junction, and results in flaccid paralysis. Current treatment relies on antitoxins that prevent entry of the toxin into cells but is ineffective against toxin that has already entered neurons. Therefore, additional therapeutics need to be identified. BoNT/A, which targets SNAP-25, has the most long-lasting effect of the seven BoNT serotypes. Our path to a high content assay for inhibitors of BoNT/A began with the identification of a highly sensitive model system, primary cultures of embryonic chick motor neurons. These cultures are relatively easy to obtain and Western blot analysis confirmed more than 50% cleavage of SNAP-25 after only 3 h exposure. Numerous inhibitors were successfully tested by Western blot, but progress was limited by the constraints of the technique. Subsequently, antibodies were generated to the BoNT/A SNAP-25 cleavage site that recognize intact, but not cleaved, SNAP-25. One of these antibodies, in conjunction with an antibody that recognizes both intact and cleaved forms of SNAP-25, was used to develop a rapid 96 well format In Cell Western assay. Because this assay relies on IR range immunofluorescence, the concept was easily transitioned to fluorophores more amenable to high content screening. A CellMask stain was added to define, and limit measurement to, cell regions. We have been able to demonstrate a clear dose-response effect of BoNT/A by this method and believe that this method will ultimately lead to identification of compounds with therapeutic value against BoNT intoxication.