Young Scientist Award

Validation of an animal replacement assay for in-vitro diagnostics of botulism in humans

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Introduction: Botulism is a rare and potentially fatal disease caused by botulinum neurotoxins (BoNTs), that are produced by Gram-positive, anaerobic, spore-forming bacteria of the genus *Clostridium*. BoNTs bind to specific receptors on the presynaptic membrane of the neuromuscular junction and are internalized into recycling endosomes. After translocation of the toxin"s enzymatic subunit into the cytoplasm, it cleaves specific proteins of the SNARE-complex at positions unique for each BoNT-serotype. Five BoNT serotypes are known to cause botulism in humans (BoNT/A, B, E, F and HA). The serotypes can be further subdivided into more than 40 subtypes. The resulting within-serotype molecular variability heavily challenges BoNT detection. Until now, the gold standard for botulism diagnostics is still the mouse bioassay (MBA), which poses a heavy burden on the animal and is therefore ethically questionable.

Objectives: Previously, we established a prototype suspension array based on the Luminex® technology, which includes enrichment of the toxins via their receptor binding domain and subsequent detection of enzymatic activity using neoepitope-specific monoclonal antibodies. The aim of this work was to further develop this method to sensitively detect all BoNT sero- and subtypes pathogenic to humans in complex matrices (e.g. serum, food).

To this end the assay performance parameters based on two different toxin enrichment strategies – via monoclonal antibodies or via recombinant endogenous receptors – for BoNT/A and BoNT/B were determined: limit of blank (LoB), limit of detection (LoD) and quantifiable range.

Results: We could show that the new *in-vitro* assay was able to detect toxin concentrations down to 3 pg/ml BoNT/A1 and 1,2 pg/ml BoNT/B1 in buffer depending on the enrichment strategy. Quantification of BoNT/A1 was possible from 8 to 2000 pg/ml and for BoNT/B1 from LoD to 800 pg/ml. Therefore, the assay is able to detect toxin amounts equal or lower than the MBA. Ongoing work is addressing the comprehensive validation of the assay for BoNT/A and BoNT/B spiked into serum and food matrices.

Conclusion: In summary the here presented multiplex suspension array has the potential to replace the MBA for diagnostics of botulism in humans. The main advantage over other suggested replacement methods is that it is a robust, highly sensitive assay that can be performed with commonly available laboratory equipment ensuring a broad applicability in routine laboratories.