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THE BINACLE (BINDING AND CLEAVAGE) ASSAY FOR THE *IN VITRO* DETERMINATION OF BOTULINUM NEUROTOXIN ACTIVITY

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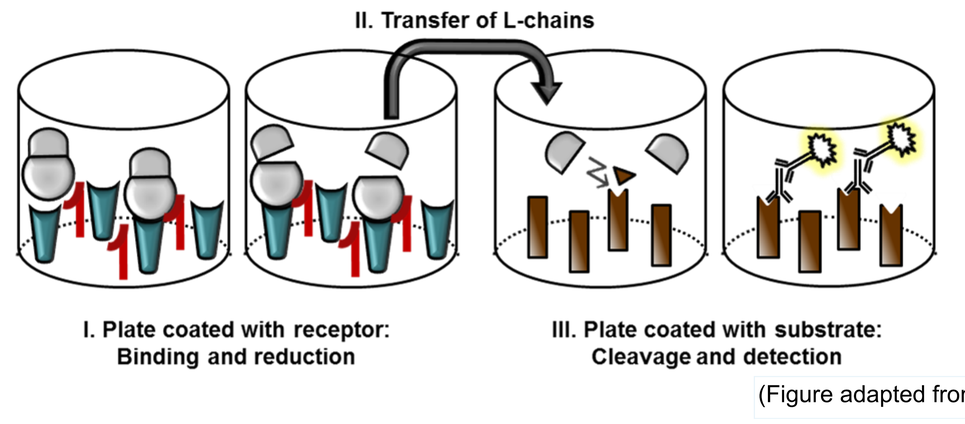
1. INTRODUCTION

Based on their muscle-relaxing effects, the botulinum neurotoxin (BoNT) serotypes A and B are widely used in clinical and aesthetic medicine. For each batch of these pharmaceutical toxin products, an exact potency determination is required to avoid undesirable side effects. Originally, these potency measurements were based on toxicity testing in mice. In line with the European directive 2010/63/EU, a replacement by an animal-free method would be highly desirable. To date, no reliable *in vitro* method for potency determination exists which is applicable to all relevant BoNT products and freely available for all potential users.

We have developed the **BINACLE (binding and cleavage) assay** that reliably measures the activity of BoNT/A or BoNT/B *in vitro* based on their two most important specific characteristics: The receptor-binding ability of the heavy (H-) chain and the proteolytic activity of the light (L-) chain [1;2]. Validation studies are ongoing to examine the suitability of this BINACLE assay as an alternative method for the potency testing of pharmaceutical BoNT products.

Aim: Replacement of the BoNT toxicity tests in mice.

2. METHOD

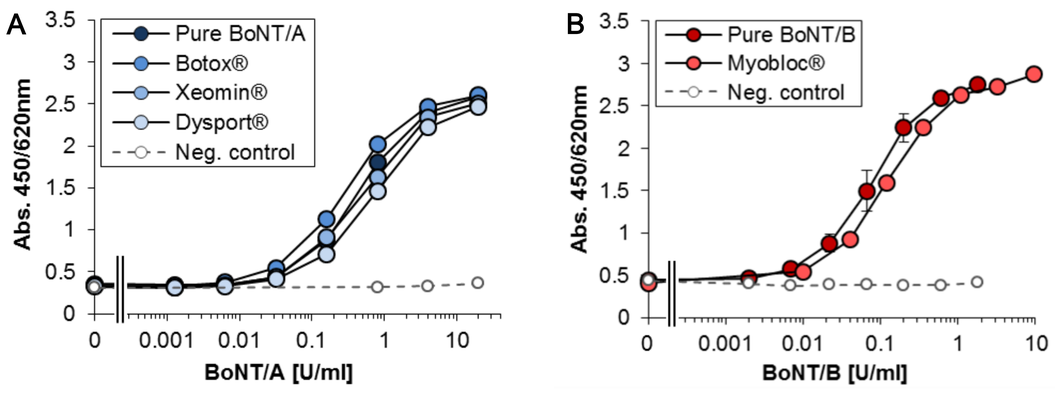


(Figure adapted from [3])

Principle of the BINACLE assay. (I.) BoNT molecules (grey) bind to immobilized receptors (blue and red) via their H-chains. Then the L-chains are released by reduction of the disulfide bonds. (II.) The supernatant with the liberated L-chains is transferred to a plate containing an immobilized substrate protein (brown). (III.) BoNT L-chains cleave the substrate protein, and the cleavage fragment is detected using a specific antibody.

3. IN-HOUSE VALIDATION

- The BINACLE assay can measure the activity of diverse BoNT/A and BoNT/B preparations containing either pure toxin or BoNT complexes
- The method is applicable to the approved BoNT/A and BoNT/B products
- Already toxin concentrations <1 U/ml BoNT are detectable

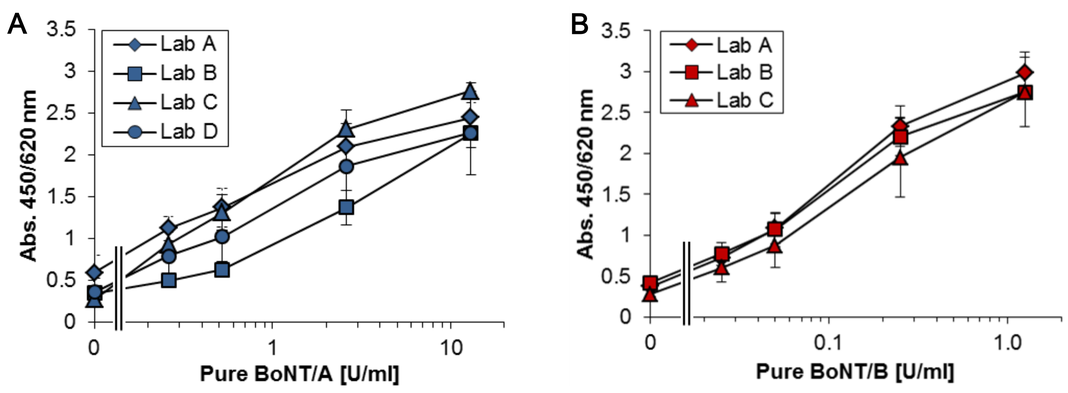


Activity measurements of various BoNT products in the BINACLE assay. Toxin concentrations are indicated in mouse LD_{50} units per ml. Each symbol represents the mean value of a triplicate measurement; error bars show the standard deviation. Small error bars are masked by the corresponding symbols. Negative controls (open symbols) represent unspecific absorbance signals measured after incubation of toxin samples in wells without receptor molecules. (A) Pure BoNT/A for research use and the pharmaceutical BoNT/A products Botox®, Xeomin®, and Dysport® were applied to the BoNT/A BINACLE assay. (B) Pure BoNT/B for research use and the pharmaceutical BoNT/B product Myobloc® were applied to the BoNT/B BINACLE assay.

4. TRANSFERABILITY STUDY

Identical BoNT-containing samples were tested in the BINACLE assay in 4 laboratories in parallel: Labs A-C each performed 6 independent tests (3 for BoNT/A, 3 for BoNT/B); Lab D performed 3 independent tests for BoNT/A only.

- All participating laboratories performed the BINACLE assay successfully
- A clear dose-response relationship was obtained in each test
- For most samples, inter-laboratory variability did not exceed 30%



Results of the transferability study. The x-axes represent the toxin concentration; the y-axes represent the absorbance measured in the BINACLE assay. Each symbol shows the mean and standard deviation of the signals that were measured for the corresponding sample in the 3 test runs performed by the respective participant. Labs A, B, C, and D are represented by diamonds, squares, triangles, and circles. (A) Results for samples containing pure BoNT/A. (B) Results for samples containing pure BoNT/B.

5. CONCLUSION

The BINACLE assay is sensitive, specific, and applicable to all approved BoNT products.

The assay protocol can be straightforwardly transferred to other laboratories.

Further validation studies are planned to pave the way for a regulatory acceptance of the BINACLE assay for the potency testing of BoNT products.

Final aim is to introduce the method into the European Pharmacopoeia, and thus to promote the replacement of the LD50-based BoNT potency test in mice.

6. OUTLOOK

An **international collaborative trial** is currently initiated to promote the regulatory acceptance of the BINACLE assay. Key features of this collaborative trial:

- ~10-12 participants (manufacturers, authorities, research laboratories); recruitment of participants is ongoing
- Participants are provided with protocols, test reagents and BoNT samples
- Each participant performs ~6 BINACLE assays
- Results will be presented to relevant committees (e.g. European Pharmacopoeia expert groups)

References

[1] Wild E et al. Toxicology In Vitro 34 (2016) 97-104
[2] Behrensdorf-Nicol HA et al. Toxicology In Vitro 53 (2018) 80-88
[3] Behrensdorf-Nicol HA et al. ALTEX 32 (2015) 41-46

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