
Poster: strategies to reduce animal numbers for testing biologicals

A combined assay measuring binding and enzymatic activity allows *in vitro* detection of tetanus toxicity in vaccines

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Tetanus neurotoxin (TeNT) consists of two disulfide-linked subunits. The heavy chain mediates the binding to neurons, whereas the light chain cleaves the neuronal protein synaptobrevin and thus causes the spastic paralysis characteristic of tetanus infections. Formaldehyde-inactivated TeNT preparations (tetanus toxoids) are used as vaccines. According to the European Pharmacopoeia, these toxoids have to be tested for “Absence of toxin and irreversibility of toxoid” by injecting them into guinea pigs and observing the animals for tetanus symptoms. Our aim is to develop an *in vitro* method which can replace these animal tests. First we examined whether an endopeptidase assay can be used to detect active TeNT in toxoids. However, we found that most toxoids contain high synaptobrevin-cleaving activities caused by TeNT light chains with intact protease domains. As these molecules lack a functional heavy chain, they are not toxic *in vivo*. Thus, the endopeptidase assay alone cannot reliably detect tetanus toxicity in toxoids. We are now developing a combined method consisting of a ganglioside binding and an endopeptidase activity step. Both steps are functionally linked, so that only molecules which display both activities on separate, disulfide-linked subunits generate a signal. We demonstrate that this combined assay is able to detect toxic TeNT in spiked toxoid samples.

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