Polymerase Chain Reaction (PCR) as alternative method to mouse bioassay for typing of botulinum neurotoxin-producing clostridia.

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Botulism is a severe neuroparaliytic disease characterized by flaccid paralysis. Seven antigenically distinct toxin types (A, B, C_1, D, E, F and G) of the botulinal neurotoxins (BoNTs) have been identified [1]. Although BoNTs are classically produced by *Clostridium botulinum*, since 1979 other BoNT-producing species have been isolated. In particular, *Clostridium baratii*, which produces BoNT type F, has been isolated in the United States and in Hungary *Clostridium butyricum,* which produces BoNT type E, has been isolated in Italy, in China and in India. [2, 3]. Since different species of Clostridia have the ability to produce BoNTs, conventional isolation and identification of BoNTs-producing clostridia cannot rely solely on the basis of biochemical characteristics.
Currently, microbiological methods take into consideration only *C. botulinum* species, and the identification procedure included the confirmation and typing of the BoNTs production of the strain by mouse bioassay [4]. This technique is highly sensitive and specific, but costly, time-consuming, laborious, raises ethical concern due to the use of experimental animals, and does not take in consideration other BoNTs-producing clostridia. Moreover, efforts have been made to develop alternative methods to animal testing, as international legislation recommends (Directive 86/609/EEC).
The International Organization for Standardisation (ISO) has underlined the need to harmonize and standardize PCR-based methods to detect BoNTs-producing clostridia (Resolution 259 taken during the 24th Meeting of ISO TC34 SC9 – Warsaw – June 2005). The development of molecular biological methods based on the detection of BoNTs genes would be ideal. Different PCR methods have been described for detection of BoNTs-producing clostridia in food and clinical samples, and results obtained using PCR assays to detect neurotoxin gene fragments show a very high level of agreement with those from the mouse bioassay [5]. In our experience, where the typing of BoNTs-producing clostridia has been performed for two years using multiplex PCR and the confirmation of the production of the toxins using mouse bioassay halved the use of the animals in our laboratory. During this period we have analyzed about 400 samples (clinical and food) using a modified multiplex conventional PCR method and the all the results obtained were correctly confirmed by mouse bioassay.

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