Evaluation of DNA Extraction Methods Suitable for PCR-based Detection and Genotyping of Clostridium botulinum

Sufficient quality and quantity of extracted DNA is critical to detecting and performing genotyping of Clostridium botulinum by means of PCR-based methods. An ideal extraction method has to optimize DNA yield, minimize DNA degradation, allow multiple samples to be extracted, and be efficient in terms of cost, time, labor, and supplies. Eleven botulinum toxin–producing clostridia strains and 25 samples (10 food, 13 clinical, and 2 environmental samples) naturally contaminated with botulinum toxin–producing clostridia were used to compare 4 DNA extraction procedures: Chelex® 100 matrix, Phenol-Chloroform-Isoamyl alcohol, NucliSENS® magnetic extraction kit, and DNeasy® Blood & Tissue kit.

Integrity, purity, and amount of amplifiable DNA were evaluated. The results show that the DNeasy® Blood & Tissue kit is the best extraction method evaluated because it provided the most pure, intact, and amplifiable DNA. However, Chelex® 100 matrix seems to be suitable for PCR-based methods intended for laboratory diagnosis of suspected outbreaks of botulism, because it is faster and cheaper compared to DNeasy® Blood & Tissue kit, and for samples in which the mean of Ct values obtained are statistically different (P>0.05) with respect to the best method, no lack of PCR amplification was shown. In addition, molecular methods for laboratory diagnosis currently are based on a microbial enrichment step prior to PCR, and so the differences in amplification seem to not influence the analytical results.