

## ***CLOSTRIDIUM BOTULINUM***

### ***CLOSTRIDIUM BOTULINUM* CELL NUMBERS FOR DETECTABLE BOTULINUM TOXIN PRODUCTION IN BROTH AND FOODS**

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The environmental factors affecting spore germination, growth and toxin production of *C. botulinum* are well documented. However, the number of *C. botulinum* vegetative cells at which botulinum toxin is detectable either in broth or foods has not been determined. Hence, the objective of this study was to determine vegetative cell numbers of *C. botulinum* present when toxin is initially detectable by the mouse bioassay.

Spores of two strains each of proteolytic or non-proteolytic *C. botulinum* strains were inoculated into Trypticase-peptone-glucose-yeast (TPGY), and mashed potatoes for proteolytic strains and canned tuna for non-proteolytic strains. Two inoculation levels (10 and 1000 spores/ml) and two incubation temperatures (15° and 31°C for proteolytic strains or 12° or 31°C for nonproteolytic strains) were used for the broth study to determine the effect of inoculum size and temperature on vegetative cell numbers of *C. botulinum* present when toxin is detectable. Studies with foods were conducted at 31°C with an inoculation level of 1000 spores/g. Inoculated samples were sampled every hour (in broth) or two hours (in foods) near toxin production times as determined in a preliminary study. At each sampling time, *C. botulinum* cell numbers in the samples were determined by plating, whereas botulinum toxin production was determined by the mouse bioassay. Results revealed that *C. botulinum* cell numbers were at  $10^5$  to  $10^8$  cfu/ml or g when toxin was first detected. Spore inoculation level (10 v. 1000 spores/ml or g) had little influence on cell numbers when toxin was initially detectable; however, a longer incubation time was needed for low level inoculum to grow and produce detectable toxin. Incubation temperatures (31°C vs. 12° or 15°C) had little influence on cell numbers when toxin was first detected; however, a longer incubation time was needed at low temperatures to reach detectable toxicity. Proteolytic *C. botulinum* cell numbers were higher in broth than in foods when toxin was initially detected. Longer incubation times were needed for foods than broth to reach detectable toxicity for both types of *C. botulinum* strains. Non-proteolytic strains produced toxin at earlier sampling times than proteolytic strains, both in broth and foods.