CLOSTRIDIUM BOTULINUM

THE ORGANISM/TOXIN

Clostridium botulinum is one of the most important pathogens associated with food. The organism forms spores that are resistant to many common food process controls. Botulinal neurotoxins (BoNT) produced by vegetative cells of this Gram positive, anaerobic bacterium are among the most potent biological neurotoxins known.

Foodborne botulism is a very severe intoxication, historically caused by eating preserved low acid, low oxygen foods (e.g. canned vegetables, meat and fish) in which *C. botulinum* had grown and produced BoNT. Symptoms appear between 12 and 36 hours after consuming the contaminated food, with early nausea, vomiting and diarrhoea followed by paralysis of the eyes, mouth, throat and, progressively, muscles. Infant botulism is an extremely rare toxico-infection that occurs when *C. botulinum* grows and produces toxins in the intestines of babies; symptoms appear in 3-30 days and include constipation, lethargy, floppiness and breathing difficulties.

Not all *C. botulinum* cause illness in humans. Strains produce one of seven known types of BoNT (A to G). Only those producing types A, B, E and F (rarely) cause botulism in humans (WHO, 2002). Strains are also separated into two groups based on physiological differences: Group I (can produce A, B or F toxin) are proteolytic and cause food spoilage; Group II (can produce B, E or F toxin) are non-proteolytic and may be present in foods without obvious spoilage.

This datasheet expands on foodborne and infant botulism caused by *C. botulinum* in Groups I and II.

GROWTH AND ITS CONTROL

Growth

Atmosphere

Normally grows in the absence of oxygen. Growth rate is reduced under 100% CO_2 (Gibson *et al.,* 2000).

Other conditions

Condition	Group I (Toxins A, B, F; proteolytic, mesophilic)			Group II (Toxins B, E, F; non-proteolytic, psychrotrophic)		
	Minimum	**Optimum**	**Maximum**	**Minimum**	**Optimum**	**Maximum**
Vegetative cells						
Temperature	10°C	35-40°C	48°C	3.0°C	18-25°C	45°C
pH	4.6			5.0		
Water activity (a_w)	0.94 (10% NaCl)			0.97 (5% NaCl)		

Data from Johnson (2007)

Survival

Condition	Group I (Toxins A, B, F; proteolytic, mesophilic)	Group II (Toxins B, E, F; non-proteolytic, psychrotrophic)
Temperature	**Spores and toxins:** resistant to freezing temperatures as used for food storage [(1)] **Vegetative cells:** undefined	
pH	**Spores:** survive <4.6 **Toxin:** stable at low pH	**Spores:** survive <5.0 **Toxin:** stable at low pH

[(1)] From ICMSF (1996).

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Inactivation
These data are generally derived under optimal laboratory conditions. A combination of factors results in greater inhibition of growth than individual factors.

Condition	Group I (Toxins A, B, F; proteolytic, mesophilic)	Group II (Toxins B, E, F; non-proteolytic, psychrotrophic)
Vegetative cells		
Temperature	Killed by a few minutes exposure to 60ºC	
pH	<4.6 (sporulation)	<5.0 (sporulation)
Spores		
Temperature	$D^{(1)}_{100°C}$ = 25 min $D_{121°C}$ = 0.1-0.2 min [(2)]	$D_{100°C}$ = <0.1 min $D_{121°C}$ = <0.001 min [(3)]
pH	Spores of type A toxin producers: Low pH (<5.0) or high pH (>9.0) reduce D values	Not defined
Radiation	All spore types relatively resistant. In frozen foods, D = 2.0 to 4.5 kGy [(4)]	
Toxin		
Temperature	These proteins are relatively sensitive to heat. Inactivation is non-linear with small proportion heat-resistant. $D_{74°C}$ = <3 minutes in various substrates and pH values (for types A, B and E). Tomato soup anomalous at 3 minutes (pH 4.2). A 3-$D_{74°C}$ process in tomato soup for type A toxin = 25 minutes. Toxins are slightly more heat stable at lower pH values [(5)]	
pH	Inactivated rapidly at pH 11	
Radiation	Toxins are not inactivated by the doses used in food preservation	

(1) D = Time (minutes) to reduce a population by 90% at a given temperature.
(2) A 12-D reduction (equivalent to 121°C for 3 min.) known as the "botulinum cook" is universally used in canning of low-acid foods (pH>4.6). Since high-acid foods (pH<4.6) do not support germination and growth of Group I spores, the "botulinum cook" is not necessary. Group I spores are considerably more heat resistant than Group II spores.
(3) A 6-D reduction, equates to 90°C for 10 min. is generally considered sufficient for destruction of Group II spores.
(4) D values for radiation may be lowered by the presence of O_2, preservatives or temperatures above 20°C. Doses used in food preservation do not effectively eliminate the organism.
(5) From ICMSF (1996).

Preservatives
The inhibitory effect of preservatives is not sufficient as a sole control measure, but useful as a hurdle. The interactions of combinations of various preservatives used in hurdle technology are complex and only examples are given here.

Combinations of sodium/potassium nitrite and nitrate and other hurdles are used to control clostridia. Their antimicrobial effect comes from nitrous acid formation and is influenced by pH. In New Zealand, these compounds can be added to bacon, ham, saveloys, luncheon meat, salami, corned silverside, and hamburger (maximum 125 mg of total nitrite and nitrate per kg). Nitrite or nitrate are not permitted in sausage, sausage meat and mince. Nitrates can be added to cheese and cheese products (50 mg/kg maximum). The use of sorbic acid and its salts in combination with low nitrite levels are also used as controls for *C. botulinum.* (Schedule 1, Standard 1.3.1, FSANZ).

Lactic acid bacteria added in meat product starter cultures (such as salami) inhibit growth of *C. botulinum* by producing organic acids and bacteriocins. The bacteriocin nisin has recently been approved for use in various foods in New Zealand (Schedule 1, Standard 1.3.1, FSANZ).

Disinfectants / Sanitisers
Hydrogen peroxide vapour at 355 ppm inactivated spores of toxigenic clostridia, with D-times of less than two minutes Johnston *et al*., 2005). Chlorine used at low pH is more effective than at neutral or alkaline values (ICMSF, 1996). Ethylene oxide is effective at inactivating spores although other factors such as temperature impact on efficacy.

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CLINICAL PICTURE

Botulism has five forms: foodborne, infant, adult infectious, wound and inadvertent (WHO, 1999). Food may be a vehicle in the first three forms and the mechanism can be intoxication (ingestion of pre-formed BoNT) or toxico-infection (toxin produced during spore germination in the intestine). The latter is associated with infant and adult infectious botulism. Botulism intoxication and toxico-infection are difficult to recognise and relatively rare, so misdiagnosis may occur..

Treatment: Infection and toxico-infection are treated similarly by inactivating and removing BoNT as early as possible. Drugs cannot reverse the effects of BoNT. Antiserum injections neutralise circulating toxin, and stomach and intestinal contents are cleared. Subsequent treatment is supportive and includes mechanical ventilation to counteract respiratory failure (Szabo and Gibson, 2003; Johnson, 2007; WHO, 1999).

Intoxication

Incubation: Generally 12 to 36 hours, but may be several days.

Symptoms: Range and severity vary. Initial symptoms include nausea, vomiting, and diarrhoea. Neurological symptoms follow, beginning with cranial nerve areas including eye, throat and mouth, and then travelling down the body paralysing motor nerves. Lack of muscle co-ordination, fatigue and respiratory impairment are characteristic. Constipation may develop after onset of neurological symptoms; abdominal pain may be present throughout (Szabo and Gibson, 2003; Johnson, 2007; WHO, 1999).

Condition: Foodborne botulism (confirmed by detection of toxin in patient's serum (Szabo and Gibson, 2003)). Neurotoxins, the cause of illness, are produced in food by vegetative cells of *C. botulinum*.

Dose: The minimum toxic dose of BoNT in humans is unknown. Estimates of Type A toxin to cause death (in adults) are between 0.1 and 1.0 µg, extrapolated from mouse models (ICMSF, 1996). Type B toxin is similar. Toxin types E and F are approximately 10 µg (Bell and Kyriakides, 2000).

At Risk Groups: All persons.

Long Term Effects: Most cases (up to 80%) require hospitalisation for a 4-5 week period. All BoNT interfere with neurotransmitters, a temporary condition eventually restored by motor endplate regeneration. Effects are not usually long term. Toxin and vegetative cells can be excreted long after recovery (Midura 1996; Turner *et al.,* 1978). Fatalities are generally caused by respiratory failure and/or obstructed air passages. The mortality rate for foodborne botulism is approximately 10% (ACMSF, 2005).

Toxico-infection

Incubation: 3 to 30 days. Illness can result from a single exposure (WHO, 1999).

Symptoms: First sign is 3 days or more of constipation followed by lethargy, inability to feed, floppiness and respiratory compromise. Infant botulism has been reported to account for around 5% of sudden infant death syndrome cases in the USA and Europe. Severity spectrum ranges from asymptomatic (little toxin absorbed) to sudden death.

Condition: Infant and adult infectious botulism are also known as 'intestinal toxaemia botulism' and are confirmed by *C. botulinum* cells or BoNT in stools or enema fluids (Szabo and Gibson, 2003). The infant form is also known as floppy baby syndrome. Neurotoxins are produced in the large intestine by germinating spores of *C. botulinum*.

Dose: 10-100 spores in infants (estimate from honey-attributed outbreak) (Arnon *et al.,* 1979; 1992; Midura *et al.,* 1979).

At Risk Groups: Adults with radically altered intestinal microflora/major intestinal complications. Infants from 1 week to 12 months old.

Long Term Effects: Infant botulism cases require intensive supportive care. Mortality is approximately 5% (ACMSF, 2005).

SOURCES

Spores of *C. botulinum* are found in raw foods derived from soil or marine environments (overseas, to a lesser extent in New Zealand). Intoxication botulism can result when food processing failure and/or temperature abuse allows germination of spores and proliferation of vegetative cells.

Human: *C. botulinum* is not a normal part of the healthy human intestinal flora.

Animal: When animals suffer botulism it is usually caused by ingestion of pre-formed toxin (Type C and D). Predator and scavenger vertebrates that feed on carrion are believed to have developed immunity by natural selection (ICMSF, 1996).

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Food: Intoxication outbreaks have been associated with food processing failures and/or temperature abuse allowing outgrowth of the spore and toxin production. Foods implicated are those stored under oil, native fermented foods, home preserves when "bottled" or "canned", commercially canned foods, seafood and fruits and vegetables (particularly those in close contact with soil). Of 405 botulism events in the USA between 1950 and 2005, 92% were linked to home-processed foods. Honey may carry spores and has been associated with a number of cases of infant botulism (Gilbert et al., 2006). There are no known New Zealand surveys of C. botulinum in foods.
Environment: *C. botulinum* spores are found worldwide distributed in soils, dust and sediments, but at very low concentrations. Strains producing BoNT types A, B, C, D and F are generally soil and dust organisms. Type B-, C- and D-producing strains have also been recovered from marine sediments (Fletcher *et al.,* 2008). Type E-producing strains are marine inhabitants. In New Zealand, Fletcher *et al*., (2008) collected 501 harbour and inshore coastal marine sediments. No toxin was detected by the mouse bioassay method. However the molecular method (PCR) detected Type A BoNT-producing genes in one sample. Type A-producing strains are not considered marine organisms, and may have been washed downstream from a terrestrial source. A previous New Zealand environmental survey based on sediments from lakes, oxidation ponds and tidal waters around Auckland, detected C- and D-producing strains at samples from 11/20 sites (Gill and Penney, 1982). Two reasons have been proposed for the apparent lack of Type E-producing strains in the New Zealand marine environment: geographical isolation and the consistently high salinity of New Zealand waters (>3%), which may prevent the organism from establishing (Fletcher *et al.,* 2008).
Transmission Routes: Most transmission is foodborne. Person-to-person transmission does not occur.

OUTBREAKS AND INCIDENTS

NZ Incidence: Only two confirmed intoxication cases (Type A) are known to have occurred in New Zealand. These were associated with home-preserved tiroi (watercress and boiled mussels) (Flacks, 1985). No known cases of infant botulism have occurred in New Zealand during the past 20 years.

Outbreaks
These are examples of foodborne intoxication.

New Zealand, watercress and mussels (tiroi): 1984, Type A, 2 cases, both hospitalised. Boiling the mussels rather than steaming may have destroyed fermentation organisms, thereby reducing lactic acid production (Flacks, 1985; Hudson *et al*., 2001).

Canada/USA, chopped garlic stored in oil: 1985, Type B, 36 cases (Canada). 1989, Type A, 3 cases (USA). Contaminated raw garlic, stored at room temperature under anaerobic conditions without controlling factors (pH or salt).
UK, hazelnut yoghurt: 1989, Type B, 27 cases, 1 death. Sugar was changed to aspartame in the hazelnut puree formula. Altered a_w from 0.90 to 0.99.
Italy, tiramisu: 1996, Type A, 8 cases, 1 death. Temperature abuse of mascarpone cheese, an ingredient.
Georgia/USA/Canada, pasteurised carrot juice: 2006, Type A, 6 cases. No controlling factors (sugar, salt or preservative) coupled with inadequate refrigeration (Sheth *et al*., 2008).
USA, dried salted fish: 1987, Type E, 8 cases, 1 death. Uneviscerated salt-cured and air-dried. Subjected to temperature abuse.
USA, canned chilli sauce: 2007, Type A, 4 cases. Canning process deficiencies were identified.
USA**, baked potato:** 1994, 30 cases. Spores survived cooking, foil-wrap provided a reduced oxygen atmosphere, subsequent ambient storage led to toxin production. The potatoes were used to make two different dips.

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