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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

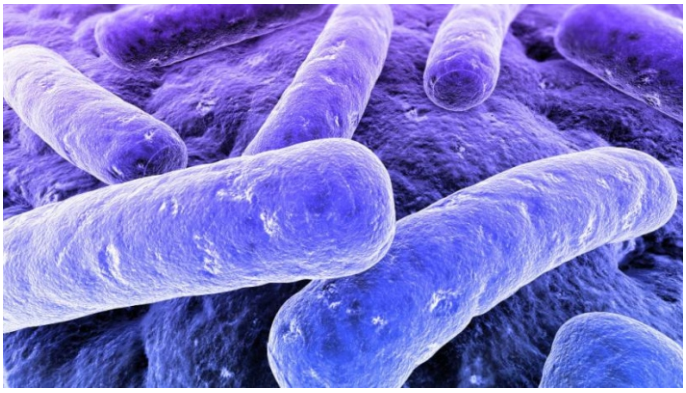
Botulism is a rare and potentially fatal illness caused by botulinum toxin, which is produced by the bacterium *Clostridium botulinum*. The disease begins with weakness, blurred vision, feeling tired, and trouble speaking. This may then be followed by weakness of the arms, chest muscles, and legs. Vomiting, swelling of the abdomen, and diarrhea may also occur. The disease does not usually affect consciousness or cause a fever.

Botulism can occur in several ways. The bacterial spores which cause it are common in both soil and water and are very resistant. They produce botulinum toxin when exposed to low oxygen levels and certain temperatures. Foodborne botulism happens when food containing the toxin is eaten. Infant botulism instead happens when the bacterium develops in the intestines and releases the toxin. This typically only occurs in children less than one year old, as protective mechanisms against development of the bacterium develop after that age. Wound botulism is found most often among those who inject street drugs. In this situation, spores enter a wound, and in the absence of oxygen, release the toxin. The disease is not passed directly between people. Its diagnosis is confirmed by finding the toxin or bacteria in the person in question. The "DISEASE **DIAGNOSIS"** segment delves deep into this subject.

"UNDERSTANDING" portion generally outlines anerobic infections caused by anaerobes in mankind. While "**TROUBLESHOOTING"** talks about problems faced in GRAMs Staining. **"BOUQUET** "can be searched within.

DISEASE DIAGNOSIS

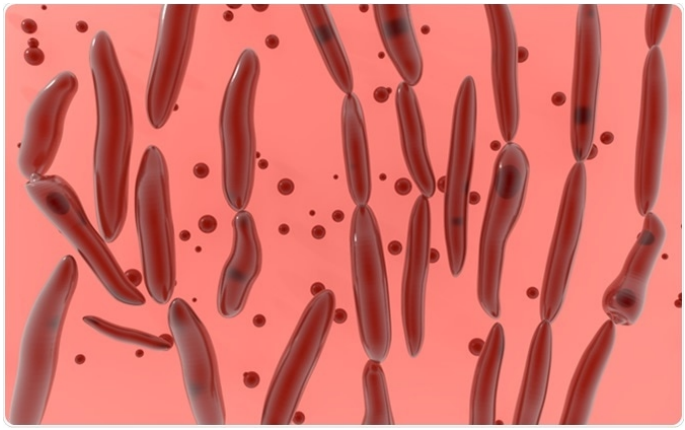
BOTULISM



Background

Botulism is a critical neurologic syndrome characterized by acute neuroparalytic manifestations resulting from a neurotoxin secreted by *Clostridium botulinum*. The toxin binds irreversibly to the presynaptic membranes of peripheral neuromuscular and autonomic nerve junctions. Toxin binding blocks acetylcholine release, resulting in weakness, flaccid paralysis, and, often, respiratory arrest. Cure occurs following sprouting of new nerve terminals. The 3 main clinical presentations of botulism include infant botulism or intestinal botulism, foodborne botulism, and wound botulism. Iatrogenic botulism also may ccur via cosmetic or therapeutic injection of any commercially made botulinum toxin (eg, Botox, Dysport, Xeomin, Myobloc). Additionally, because of the potency of the toxin and ease of aerosolization, the possibility of inhalational botulism as a bioterrorism agent or biological weapon is of great concern. For more information, see CBRNE – Botulism.

Pathophysiology

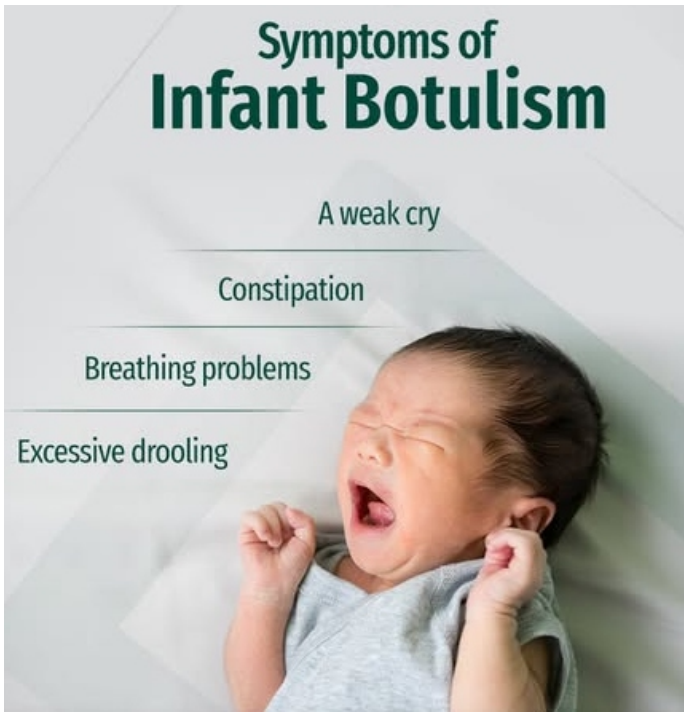


Clostridium botulinum produces 8 distinct neurotoxins, including types A through G and the potent F/A Hybrid. Among these, types A, B, E, and occasionally F and F/A Hybrid (previously known as H) can impact human health. These botulinum toxins are highly toxic proteins that can withstand degradation from stomach acid and proteolytic enzymes. Type F/A Hybrid is considered the most potent toxin among them. In the United States, around 50% of foodborne outbreaks are attributed to type A toxin, followed by types B and E. Geographically, type A toxin is more prevalent in areas west of the Mississippi River, type B is common in eastern states, and type E often is associated with regions such as Alaska and the Great Lakes area where ingestion of fish and fish products is frequent. The mechanism of action involves toxin-mediated blockade of neuromuscular transmission in cholinergic nerve fibers. This is accomplished by inhibiting acetylcholine release at the presynaptic clefts of the myoneural junctions. Toxins are absorbed from the stomach and small intestine, where they remain stable despite digestive enzymes. Subsequently, they are hematogenously disseminated to peripheral cholinergic nerve terminals (neuromuscular junctions, postganglionic parasympathetic nerve terminals, peripheral ganglia). The toxin is endocytosed by the neuron and then is allowed to cleave proteins essential for neurotransmitter release. The toxin does not cross the blood-brain barrier, likely secondary to its large size, however, it may be transported to the central nervous system axonally. Because the motor end plate responds to acetylcholine, botulinum toxin ingestion results in hypotonia that manifests as descending symmetric flaccid paralysis and is usually associated with gastrointestinal symptoms of nausea, vomiting, and diarrhea. Cranial nerves are affected early in the disease course. Later complications include paralytic ileus, severe constipation, and urinary retention. Humans commonly ingest *C botulinum* spores, but germination typically does not occur in the adult intestine since special conditions are required (ie, anaerobic environment, low acidity, specific amino acid, salt and sugar concentrations, and temperatures 37°F-99°F). Wound botulism results when wounds are contaminated with *C botulinum* spores. Wound botulism has developed rarely after cesarean delivery, following traumatic injury that involved soil contamination, and more commonly among injection drug users (particularly those who use black-tar heroin). The wound may appear deceptively benign. Traumatized and devitalized tissue provides an anaerobic medium for the spores to germinate into vegetative organisms and to produce neurotoxin, which then disseminates hematogenously. Symptoms develop after an incubation period of 4-13 days, with a median 6.5 days. The clinical symptoms of wound botulism are similar to those of foodborne botulism except that gastrointestinal symptoms (including nausea, vomiting, diarrhea) are uncommon.

Frequency

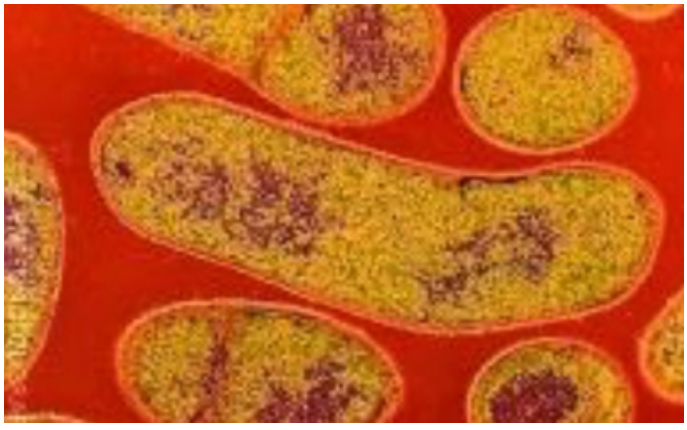
In the United States over hundreds of cases of botulism are reported annually to the Centers for Disease Control and Prevention (CDC). The latest available US surveillance summaries are from 2019. The European CDC has reported that although the annual botulism occurrence in Europe is fewer than 1 per 1,000,000 individuals, children younger than 12 months have the highest risk. Notably, based on open-source epidemic intelligence, the frequency of reported botulism cases in Ukraine has increased dramatically since the February 2022 invasion by Russia; within months of the invasion, case reporting increased by almost 400%, however, at this point the case-numbers likely are underestimated given the weakening / absence of formal reporting systems. Prior to the analysis of the increase in Ukrainian cases, Romania had reported the highest number of cases in Eastern Europe in 2014 with 31 cases. The increasing instances of *C botulinum* in Vietnam have raised alarm among healthcare authorities and policymakers due to the inadequate availability of BAT antitoxin.

Infant botulism



For 2019, the CDC reported 152 cases of infant botulism, all of which were laboratory-confirmed, with the highest case-counts coming from California and Pennsylvania. Infant botulism with mean age of 13 weeks accounts for 60-70% of all botulism cases.

Foodborne botulism



For 2019, the US CDC reported 21 cases of foodborne botulism. Outbreak-related cases are shown below:

- Alaska: Type E outbreak of 4 cases related to home-prepared fermented beluga flipper
- Colorado: Type A outbreak of 4 cases related to commercially prepared, prepackaged roasted potatoes
- Texas: Type A outbreak of 3 cases related to commercially prepared preserved peppers produced in Mexico
- China witnessed a total of 80 foodborne botulism outbreaks between 2004 and 2020, largely attributed to the excessive consumption of canned beans, tofu, and dried meat prepared with traditional techniques.
- Saudi Arabia recently reported its first foodborne botulism outbreak in 2024 from contaminated mayonnaise.
- For 2019, the CDC reported 41 cases of wound botulism; the highest number of cases overwhelmingly were reported by California. All laboratory confirmed cases occurred in persons who injected drugs and 66% of those patients reported black tar heroin use.

Wound botulism

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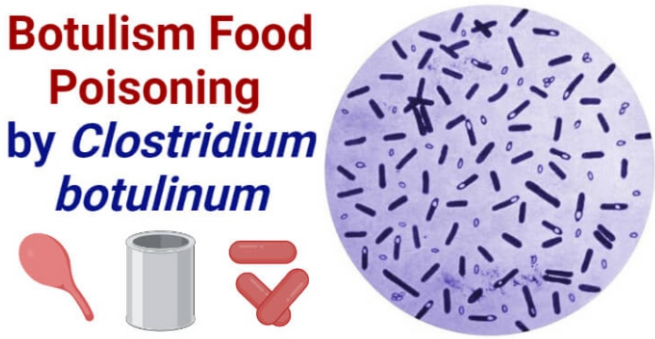
- All laboratory confirmed cases occurred in persons who injected drugs and 66% of those patients reported black tar heroin use.

Mortality/Morbidity



Mortality rates vary based on the age of the patient and the type of botulism and have significantly declined since the early 1900s due to improvements in supportive care.

Infant (Intestinal) botulism:
The risk for death due to infant botulism usually is less than 1%.

Foodborne botulism:
The modern mortality for foodborne botulism is 5% or less.

Wound botulism:
Wound botulism carries a mortality rate of roughly 10%.

Epidemiology

Sources of Botulism

Clostridium botulinum is an anaerobic, gram-positive bacterium that survives adverse conditions by forming spores and is commonly found in soil and marine sediments. Under anaerobic conditions, these spores can germinate, leading to the production of a highly potent botulinum toxin, which is the most potent toxin known on a molecular weight basis.

Infant botulism

Infant botulism is by ingested *C botulinum* spores that germinate in the infant's intestine. Sources include environmental spores from soil, dust, or contaminated food products such as honey and corn syrup. Despite the association with honey, most cases occur without known exposure to it. Clinical management primarily involves supportive care, with an infant mortality rate of less than 1%.

Foodborne botulism

Foodborne botulism typically results from ingestion of toxin in improperly canned or home-prepared food. Sources include environmental spores from soil, dust, or contaminated food products like honey and corn syrup. Despite the association with honey, most cases occur without known exposure to it. Honey, due to its low water activity (0.5 - 0.65), does not support the germination of C. botulinum spores, which require a water activity over 0.94.

Wound botulism

Occurs predominantly in adults and can be associated with intravenous drug use, as demonstrated by a case involving a 40-year-old patient who injected black tar heroin and developed botulism, requiring intensive care and antitoxin administration.

Geographic and demographic distribution

C botulinum spores are detected in approximately 20% of soil samples worldwide, with specific toxins associated with different regions.

Toxin A is found predominantly west of the Mississippi River in cases of wound botulism.

Toxin B is most common in the eastern United States, associated with infant botulism.

Toxin E is linked to northern latitudes and frequently associated with fish products.

Impact on wildlife

Toxins C and D are frequent causes of botulism in animals, particularly affecting birds and carnivores. Vultures exhibit high resistance to these toxins, whereas waterfowl are vulnerable due to their feeding habits.

Global incidence and public health implications

A comprehensive analysis of 6,932 botulism cases from 59 nations revealed a global case fatality rate of 1.37%, with significant underreporting estimated at 88.71% in 2016. The study emphasized the need for improved awareness among healthcare professionals, better global reporting mechanisms, and enhanced surveillance to reduce the incidence and improve outcomes of botulism cases worldwide.

Sex

Wound botulism is more common in females. Foodborne botulism has no sexual predilection.

Age

Foodborne botulism and wound botulism predominately occur in adults. The mean age of infant botulism is 3 months. The vulnerability of infants at the 3–5-month age is thought to be secondary to the change in bacterial taxa while transitioning to foods other than breast milk. From 1976 to 1983, California found a greater percentage of botulism patients who were breastfed versus age-matched controls; however, this discrepancy is attributed to the theory that breastfeeding delays the colonization of the infant microbiome with *C botulinum* and slows the development of life-threatening toxemia enough so that cases may be diagnosed in the hospital, rather than an infant death occurring at home and being attributed to sudden infant death syndrome (which is twice as likely to occur with formula-fed infants).

Prognosis

Prompt and vigorous supportive care, especially respiratory care, greatly improves the prognosis. The recovery period from botulism flaccid paralysis takes weeks to months. Death that occurs early in the course of disease is usually secondary to acute respiratory failure, whereas death later in the course of illness is typically secondary to complications associated with prolonged intensive care (ie, venous thromboembolism or hospital-acquired infection). Some patients demonstrate residual weakness or autonomic dysfunction for 1 year after the onset of the illness. However, most patients achieve full neurologic recovery. Permanent deficits may occur in those who sustain significant hypoxic insults.

History

Following the onset of symptoms, botulism quickly progresses over several days. The magnitude of the neuromuscular impairment can advance hourly. Persons who survive this phase eventually stabilize and then recover over a period of days to months. The mechanism of recovery is not fully understood but requires the generation of new presynaptic axons and the formation of new synapses, as the original synapses are permanently affected. As with tetanus, recovery from botulism does not confer long-term immunity. Rare reports have described a second episode in the same patient.

Foodborne botulism

Symptoms of foodborne botulism typically begin with gastrointestinal issues, appearing 18 to 36 hours after ingestion of contaminated food, though they can start as early as 2 hours or as late as 8 days. Initial symptoms include nausea, vomiting, and diarrhea, often followed by constipation. Many patients also report experiencing a dry mouth. As the illness progresses, patients may develop acute neurologic symptoms about a day after consuming the contaminated food. The severity of foodborne botulism can vary from mild to severe. If the condition worsens, death can occur, typically around 3 days after hospital admission. The modern mortality rate for this condition is 5% or less, indicating improved outcomes due to advances in medical care.

Infant botulism

The incubation period for infant botulism is 2-4 weeks. The peak age of incidence is 2-4 months. Constipation is the usual presenting symptom, often preceding motor function symptoms by several days or weeks. Other signs of autonomic dysfunction usually present early as well, including those mentioned above. Gag reflexes frequently are impaired, which can lead to aspiration if the airway is unprotected.

Wound botulism

Patients with wound botulism typically have a history of traumatic injury with wounds that are contaminated with soil. Since 1994, the number of patients with wound botulism who have a history of chronic intravenous drug abuse has increased dramatically. In most cases, black-tar heroin has been the implicated vehicle. Researchers followed 17 heroin users who had recurrent botulism after using black-tar heroin. Physicians need to be alert to recognize botulism, especially in patients who use black-tar heroin or in those with a history of injection drug–associated botulism. Rare cases of wound botulism after cesarean delivery have been documented. Aside from a longer incubation period, wound botulism is similar to foodborne botulism. The incubation period of wound botulism ranges from 4-13 days, with a median 6.5 days. Unlike foodborne botulism, gastrointestinal symptoms (including nausea, vomiting, diarrhea) are uncommon in wound botulism. Patients may be febrile, but this more likely is due to the wound infection rather than the wound botulism. In many cases, the wound appears benign.

Adult intestinal toxemia

Adult intestinal toxemia results from enteric colonization with *C botulinum* that progresses to toxin production. The pathophysiology of the changes in the gastrointestinal flora that facilitate colonization is unclear.

Iatrogenic botulism due to accidental overdose of botulinum toxin (Botox or Dysport): Cases of botulism due to Botox overdosage have been reported. Symptoms vary and can include dysphagia, ptosis, and diplopia, as well as more severe presentations of systemic weakness or muscle paralysis.

Systemic botulism after self-administered botulinum injection: A 46-year-old woman presented with respiratory difficulties, weakness, dysphagia, and gait issues a week after self-injecting 100 units of BoNT-A for cosmetic purposes. Despite developing ptosis and neck weakness, she largely retained normal limb function and cognitive abilities. Diagnosed with systemic botulism, she received pyridostigmine treatment, showed improvement, and was transferred to a community

hospital after nine days. Although she had residual mild dysphagia, her overall progress was positive, and she was discharged after a month with a full recovery in the following 2 months. The case underscores the rare yet severe effects of illicit BoNT-A injections and stresses the importance of careful administration to prevent such systemic botulism complications.

Physical

Almost all patients with foodborne or intestinal exposure are afebrile and the majority of patients will display descending paralysis with cranial nerve palsies early in the disease process. A collection of anticholinergic toxicity symptoms will present such as nausea, vomiting, and the 4 D's, dysphagia, diplopia, dysarthria, and dry mouth. Mydriasis is seen in 50% of cases.

Generally, botulism progresses as follows:

- Preceding or following the onset of paralysis are nonspecific findings such as nausea, vomiting, abdominal pain, malaise, dizziness, dry mouth, dry throat, and, occasionally, sore throat. Except for nerves I and II, the cranial nerves are affected first.
- Cranial nerve paralysis manifests as blurred vision, diplopia, ptosis, extraocular muscle weakness of paresis, fixed/dilated pupils, dysarthria, dysphagia, and/or suppressed gag reflex. Additional neurologic manifestations include symmetric descending paralysis or weakness of motor and autonomic nerves.
- Respiratory muscle weakness may be subtle or progressive, advancing rapidly to respiratory failure. Progressive muscle weakness occurs and often involves the muscles of the head and neck, as well as intercostal diaphragmatic muscles and those of the extremities.

The autonomic nervous system also is involved. Manifestations of this include the following:

- Paralytic ileus advancing to severe constipation
- Gastric dilatation
- Bladder distention advancing to urinary retention
- Orthostatic hypotension
- Reduced salivation
- Reduced lacrimation

Other neurologic findings include the following:

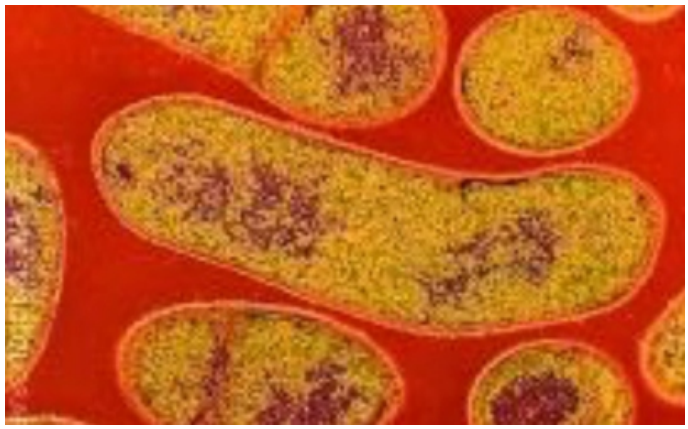
- Changes in deep tendon reflexes, which may be either intact or diminished
- Incoordination due to muscle weakness
- Absence of pathologic reflexes and normal findings on sensory and gait examinations
- Normal results on mental status examination

Causes

Infant botulism

Infant botulism arises when C. botulinum spores, ingested from environmental sources such as soil, dust, or contaminated food products like honey and corn syrup, germinate in an infant's intestine. Although honey is often linked to this condition, the majority of cases are reported without any known exposure to honey.

Foodborne botulism



Of the roughly 110 cases of botulism that occur in the US annually, foodborne exposure accounts for ~25% of cases. It results from the ingestion of preformed neurotoxins; A, B, and E are the most common. California, Washington, Colorado, Oregon and Alaska, have accounted for >50% of reported foodborne outbreaks in the US since 1950. High-risk foods include home-canned or home-processed low-acid fruits, vegetables, fish and fish products (neurotoxin serotype E). Commercially processed foods and improperly handled fresh foods are occasionally associated with botulism outbreaks. The type of food responsible for approximately 28% of outbreaks remains unknown.

Wound botulism

Wound botulism results when wounds are contaminated with *C botulinum* spores. Wound botulism has developed rarely after cesaerean delivery, following traumatic injury that involved soil contamination, and more commonly among injection drug users (particularly those who use black-tar heroin). Wound botulism illness can occur even after antibiotics are administered to prevent wound infection. Wound botulism from black-tar heroin use has primarily occurred in California.

Diagnostic Considerations

The diseases most frequently confused with botulism are those that produce generalized weakness. Differentiating botulism from other diseases is essential for early initiation of therapy. Botulism should be considered in patients who are afebrile and mentally intact and who have symmetric descending paralysis without sensory findings. The diagnosis should be suspected on clinical grounds in the context of an appropriate history. A probable case is defined as a clinically compatible case with an epidemiologic link such as the ingestion of a home-canned food within the prior 48 hours.

Other conditions on the differential when considering botulism include the following :

- Aminoglycosides: Very large doses can induce neuromuscular blockade.
- Basilar artery stroke
- Cerebrovascular disease of the brainstem
- Congenital neuropathy or myopathy
- Diphtheria
- Drugs, penicillamine
- Encephalitis
- Fisher variant of Guillain-Barré syndrome
- Guillain-Barré syndrome
- Intracranial mass lesions

- Lambert-Eaton syndrome
- Myasthenia gravis
- Neurasthenia
- Poisonings by atropine, scopolamine, organophosphate insecticides, shellfish, amanita mushrooms, carbon monoxide, methyl alcohol, methyl chloride, and sodium fluoride
- Poliomyelitis
- Progressive external ophthalmoplegia
- Tick paralysis.

WORKUP

Approach Considerations

High clinical suspicion and clinical diagnosis with immediate antitoxin administration is the cornerstone of management, as laboratory tests are not helpful in the routine diagnosis of botulism; however, they are of help in identifying outbreaks and bioterrorism. A clinical criteria tool for early diagnosis has been developed for outbreak settings. When botulism is suspected, consult public health officials immediately, request antitoxin, and if transferring to a higher level of care consider administering antitoxin before transfer. Full neurologic exams, brain imaging, lumbar puncture, electromyography, nerve conduction studies, and monitoring for anaphylaxis after antitoxin administration should be performed as applicable. WBC counts and erythrocyte sedimentation rates usually are normal. Cerebrospinal fluid is also normal, except for occasional mild elevations in protein concentration. Collect specimens early for laboratory confirmation as toxin levels decrease over time, and store and transport them at refrigeration temperatures (36°F–46°F). Conduct frequent, serial neurologic examinations focusing on cranial nerve function, swallowing ability, respiratory status, and extremity strength.

Infant botulism

Infants with botulism often experience severe constipation, necessitating an enema to collect stool samples for testing spores and toxins. The CDC advises using sterile nonbacteriostatic water for the enema to avoid interference with lab tests. Stool samples should be stored in a leak-proof container, refrigerated, and sent to the laboratory promptly. Ideally, samples should be collected before beginning treatment with immune globulin, although treatment should not be delayed for test results. Treatment with immune globulin should commence immediately based on clinical suspicion, as delays can increase the risk of mortality and morbidity. Although PCR testing for spores can provide quick results and has been successful, it is not widely available, and its sensitivity levels are not fully established. For testing guidance, contact local or state public health departments. A lumbar puncture can help rule out Guillain-Barré syndrome, which typically shows elevated protein levels in the cerebrospinal fluid, and can also exclude meningitis or encephalitis, as botulism does not increase white blood cells in the CSF.

Laboratory Studies

Laboratory confirmation of botulism requires either botulinum neurotoxin isolation or growth of a botulinum neurotoxin-producing *Clostridium* species (ie, *C botulinum, C baratii, or C butyricum*) in a stool, gastric aspirate, food, or wound culture. Botulinum neurotoxin isolation requires intraperitoneal injection of the patient's serum, fluid extract of food or feces, etc. into pairs of mice with and without monovalent antitoxin followed by observation for development of clinical botulism. This test was standardized in the 1970s and has limited sensitivity depending on the mode of toxin exposure. These assays are limited to specific state public health and CDC laboratories, where other assays may also be used to determine neurotoxin serotype. Patient samples must be collected prior to administration of antitoxin, but antitoxin administration must not be delayed in order to obtain samples (serum 5-15 mL, stool 10-20 g, gastric aspirate 5-10 mL, suspected food source 10-20 g or mL). As the "gold-standard" assay has historically been the ethically controversial mouse bioassay, which requires the use of laboratory animals and personnel trained to recognize signs of botulism in mouse over the course of 4 days, a faster and less resource-intensive technology has been produced with assistance from the US CDC for use in public health labs: the BoNT Endopep-MS method utilizes mass-spectrometry to detect toxins A, B, E, and F and only requires an 8-hour period. The use of this tool has not yet been added to the Botulism management guidelines as of August, 2024.

To send a specimen to CDC for testing

- Call CDC consultant to discuss submission of specimens through local or state health department or state public health laboratory
- Maintain the specimen at 36 F-46 F and ship with cold packs
- Label package as required for Category B substances
- Include completed CDC 50.34 with selected test order CDC-10132, Botulism Laboratory Confirmation and include phone and fax numbers for the state health department and hospital
- Send package to: STAT (Attn: Botulism Lab, Unit 26) Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Atlanta, GA 30329
- Provde a tracking number to the CDC National Botulism Laboratory

C botulinum may be grown on selective media from samples of stool or foods. Note that the specimens for toxin analysis should be refrigerated (not frozen), but culture samples of *C botulinum* should not be refrigerated. Final results from culture for *Clostridium* species may take 2-3 weeks. Because intestinal carriage is rare (and adult intestines typically do not allow for germination), identifying the organism or its toxin in vomitus, gastric fluid, or stool strongly suggests the diagnosis. Isolation of the organism from food without toxin is insufficient grounds for the diagnosis. Only experienced personnel who have been immunized with botulinum toxoid should handle the specimens. Because the toxin may enter the blood stream through the eye or via small breaks in the skin, precaution is warranted. Wound cultures that grow *C botulinum* suggest wound botulism.

Imaging Studies

Imaging studies are generally not useful in the diagnosis of botulism.

The only potential role for imaging studies (eg, CT scan, MRI) would be to rule out CNS pathology, such as intracranial mass lesions, cerebrovascular disease of the brainstem, or basilar artery stroke, in patients in whom the presentation is atypical or vague.

Other Tests

Results from nerve conduction studies are normal, and electromyography (EMG) reveals reduced amplitude of compound muscle action potentials. EMG may be useful in establishing a diagnosis of botulism, but the findings can be nonspecific and nondiagnostic, even in severe cases. Characteristic findings in patients with botulism include brief low-voltage compound motor-units, small M-wave amplitudes, and overly abundant action potentials. An incremental increase in M-wave amplitude with rapid repetitive nerve stimulation may help to localize the disorder to the neuromuscular junction. Single-fiber EMG may be a more useful and sensitive method for the rapid diagnosis of botulism intoxication, particularly in the absence of signs of general muscular weakness. The results of the edrophonium chloride, or Tensilon, test for myasthenia gravis may be falsely positive in patients with botulism. If positive, it is typically much less dramatically positive than in patients with myasthenia gravis.

MANAGEMENT

Rigorous supportive care, including use of the following, is essential in patients with botulism:

- Meticulous airway management: Of paramount importance, since respiratory failure is the most important threat to survival in patients with botulism
- Cathartics and enemas: Administered to patients with bowel sounds to remove unabsorbed botulinum toxin from the intestine
- Stress ulcer prophylaxis: A standard component of intensive care management
- Nasogastric suction and intravenous hyperalimentation: Helpful if an ileus is present; if no ileus is present, tube feeding can be used for nutritional supplementation
- Foley catheter: Often used to treat bladder incontinence; the catheter must be monitored conscientiously and changed regularly
- Antibiotic therapy: Useful in wound botulism, but has NO role in foodborne botulism
- Magnesium salts, citrate, and sulfate should not be administered, because magnesium can potentiate the toxin-induced neuromuscular blockade.

Wound botulism requires the following:

- Incision and thorough debridement of the infected wound
- Antitoxin therapy
- Penicillin G IV (metronidazole if penicillin-allergic)
- Tetanus toxoid booster

Prevention of nosocomial infections

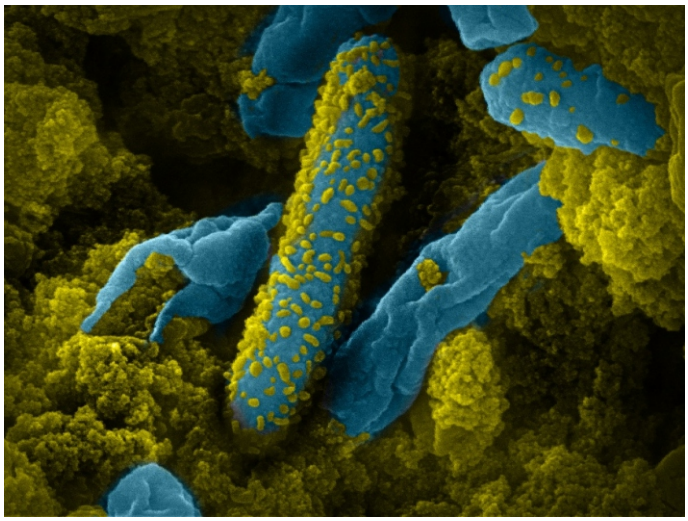
Measures to reduce the risk of nosocomial infections include the following:

- Close observation for hospital-acquired infections: Especially pneumonia (particularly aspiration pneumonia); precaution against aspiration also is necessary
- Close observation for urinary tract infection
- Meticulous skin care: To prevent decubital ulcers and skin breakdown

Careful attention to peripheral and central intravenous catheters with regular site rotation to reduce the risks of thrombophlebitis, cellulitis, and line infections should be part of the patient's supportive care.

UNDERSTANDING

ANAEROBIC INFECTIONS



Anaerobes, primarily part of the human indigenous flora, cause infections when they breach mucocutaneous barriers, often leading to abscesses, tissue necrosis, and foul-smelling discharges. Key pathogens include *Bacteroides fragilis* (abdominal infections), *Fusobacterium* (head/neck, Lemierre syndrome), *Clostridium* (gas gangrene, tetanus), and *Peptostreptococcus*. These infections are frequently polymicrobial and occur in immunocompromised patients or following tissue trauma.

Key Anaerobes and Associated Infections

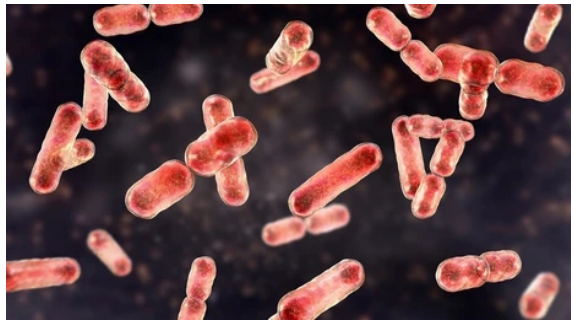
- **Gram-negative rods:** *Bacteroides fragilis* (most common, abdominal infections/abscesses), *Prevotella* (oral/soft tissue), *Fusobacterium* (Lemierre syndrome, brain abscess), *Bilophila*.
- **Gram-positive cocci:** *Peptostreptococcus* spp. (common in brain, abdominal, and skin infections).
- **Gram-positive bacilli:** *Clostridium* spp. (*C. perfringens* - gas gangrene; *C. tetani* - tetanus; *C. botulinum* - botulism), *Actinomyces* (actinomycosis), *Propionibacterium*.
- **Gram-negative cocci:** *Veillonella*.

Common Clinical Manifestations

- **Respiratory:** Aspiration pneumonia, lung abscess, chronic sinusitis.
- **Head/Neck:** Chronic otitis media, mastoiditis, dental infections, deep neck infections.
- **Abdominal/Pelvic:** Intra-abdominal abscesses, peritonitis, pelvic inflammatory disease.
- **Soft Tissue/Skin:** Necrotizing infections, bite wounds, diabetic foot infections, decubitus ulcers.
- **Systemic:** Bacteremia and endocarditis.

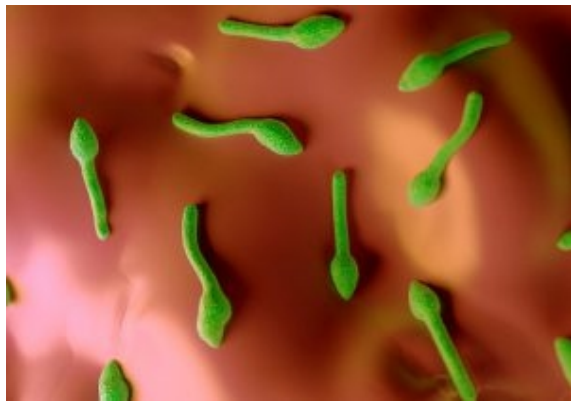
Characteristics of Infections

- **Site:** Primarily endogenous, occurring where normal flora exists (GI tract, mouth, female genital tract).
- **Putrid odor:** A foul-smelling, necrotic discharge is a strong, though not universal, diagnostic indicator.
- **Synergy:** Often coexist with facultative anaerobes (e.g., *E. coli*), which consume oxygen and facilitate anaerobic growth.
- **Treatment:** Involves drainage and antibiotics like metronidazole or clindamycin, which offer strong anaerobic coverage.

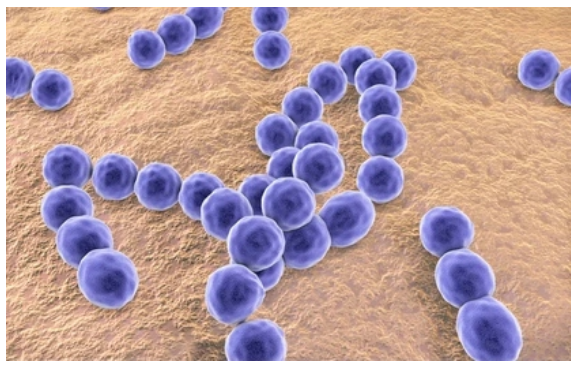


Bacteroides fragilis

Fusobacterium



Clostridium



Peptostreptococcus

TROUBLESHOOTING

The Gram Stain

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (1853-1938), who developed the technique in 1884 (Gram 1884). The importance of this determination to correct identification of bacteria cannot be overstated as all phenotypic methods begin with this assay.

The Basic Method

1. First, a loopful of a pure culture is smeared on a slide and allowed to air dry. The culture can come from a thick suspension of a liquid culture or a pure colony from a plate suspended in water on the microscope slide. Important considerations:
   - Take a small inoculum—don't make a thick smear that cannot be completely decolorized. This could make gram-negative organisms appear to be gram-positive or gram-variable.
   - Take a fresh culture—old cultures stain erratically.
2. Fix the cells to the slide by heat or by exposure to methanol. Heat fix the slide by passing it (cell side up) through a flame to warm the glass. Do not let the glass become hot to the touch.
3. Crystal violet (a basic dye) is then added by covering the heat-fixed cells with a prepared solution. Allow to stain for approximately 1 minute.
4. Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.
5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye, making it more difficult to decolorize and reducing some of the variability of the test.
6. Briefly rinse with water.
7. Decolorize the sample by applying 95% ethanol or a mixture of acetone and alcohol. This can be done in a steady stream, or a series of washes. The important aspect is to ensure that all the color has come out that will do so easily. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple with Gram-negative organisms colorless. The decolorization of the cells is the most "operator-dependent" step of the process and the one that is most likely to be performed incorrectly.
8. Rinse with water to stop decolorization.
9. Rinse the slide with a counterstain (safranin or carbol fuchsin) which stains all cells red. The counterstain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain, it's effect is only seen in the previously colorless gram-negative cells which now appear pink/red.
10. Blot gently and allow the slide to dry. Do not smear.

What's Going On?

Bacteria have a cell wall made up of peptidoglycan. This cell wall provides rigidity to the cell, and protection from osmotic lysis in dilute solutions. Gram-positive bacteria have a thick mesh-like cell wall, gram-negative bacteria have a thin cell wall and an outer phospholipid bilayer membrane. The crystal violet stain is small enough to penetrate through the matrix of the cell wall of both types of cells, but the iodine-dye complex exits only with difficulty (Davies et al. 1983).

The decolorizing mixture dehydrates cell wall, and serves as a solvent to rinse out the dye-iodine complex. In Gram-negative bacteria it also dissolves the outer membrane of the gram-negative cell wall aiding in the release of the dye. It is the thickness of the cell wall that characterizes the response of the cells to the staining procedure. In addition to the clearly gram-positive and gram-negative, there are many species that are "gram-variable" with intermediate cell wall structure (Beveridge and Graham 1991). As noted above, the decolorization step is critical to the success of the procedure.

Gram's method involves staining the sample cells dark blue, decolorizing those cells with a thin cell wall by rinsing the sample, then counterstaining with a red dye. The cells with a thick cell wall appear blue (gram positive) as crystal violet is retained within the cells, and so the red dye cannot be seen. Those cells with a thin cell wall, and therefore decolorized, appear red (gram negative).

It is a prudent practice to always include a positive and negative control on the staining procedure to confirm the accuracy of the results (Murray et al 1994) and to perform proficiency testing on the ability of the technicians to correctly interpret the stains (Andserson, et al. 2005).

Excessive Decolorization

It is clear that the decolorization step is the one most likely to cause problems in the gram stain. The particular concerns in this step are listed below (reviewed in McClelland 2001)

1. Excessive heat during fixation: Heat fixing the cells, when done to excess, alters the cell morphology and makes the cells more easily decolorized.
2. Low concentration of crystal violet: Concentrations of crystal violet up to 2% can be used successfully, however low concentrations result in stained cells that are easily decolorized. The standard 0.3% solution is good, if decolorization does not generally exceed 10 seconds.
3. Excessive washing between steps: The crystal violet stain is susceptible to wash-out with water (but not the crystal violet-iodine complex). Do not use more than a 5 second water rinse at any stage of the procedure.
4. Insufficient iodine exposure: The amount of the mordant available is important to the formation of the crystal violet – iodine complex. The lower the concentration, the easier to decolorize (0.33% – 1% commonly used). Also, QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine from solution. A closed bottle (0.33% starting concentration) at room temperature will lose >50% of available iodine in 30 days, an open bottle >90%. Loss of 60% iodine results in erratic results.
5. Prolonged decolorization: 95% ethanol decolorizes more slowly, and may be recommended for inexperienced technicians while experienced workers can use the acetone-alcohol mix. Skill is needed to gauge when decolorization is complete.
6. Excessive counterstaining: As the counterstain is also a basic dye, it is possible to replace the crystal violet—iodine complex in gram-positive cells with an over-exposure to the counterstain. The counterstain should not be left on the slide for more than 30 seconds.

Alternatives to the Gram Stain

Gram's staining method is plainly not without its problems. It is messy, complicated, and prone to operator error. The method also requires a large number of cells (although a membrane-filtration technique has been reported; Romero, et al 1988). However, it is also central to phenotypic microbial identification techniques.

This method, and it's liabilities, are of immediate interest to those involved in environmental monitoring programs as one of the most common isolates in an EM program, Bacillus spp., will frequently stain gram variable or gram negative despite being a gram-positive rod (this is especially true with older cultures). The problems with Gram's method have lead to a search for other tests that correlate with the cell wall structure of the gram-positive and the gram-negative cells. Several improvements/alternatives to the classical gram stain have appeared in the literature.

KOH String Test

The KOH String Test is done using a drop of 3% potassium hydroxide on a glass slide. A visible loopful of cells from a single, well-isolated colony is mixed into the drop. If the mixture becomes viscous within 60 seconds of mixing (KOH-positive) then the colony is considered gram-negative. The reaction depends on the lysis of the gram-negative cell in the dilute alkali solution releasing cellular DNA to turn the suspension viscous. This method has been shown effective for food microorganisms (Powers 1995), and for Bacillus spp (Carlone et al 1983, Gregersen 1978), although it may be problematic for some anaerobes (Carlone et al 1983, but also see Halebian et al 1981).

This test has the advantage of simplicity, and it can be performed on older cultures. False negative results can occur in the test by using too little inoculum or too much KOH (DNA-induced viscosity not noticeable). False positive results can occur from too heavy an inoculum (the solution will appear to gel, but not string), or inoculation with mucoid colonies. This can serve as a valuable adjunct to the tradition gram stain method (von Graevenitz and Bucher 1983).

Aminopeptidase Test

L-alanine aminopeptidase is an enzyme localized in the bacterial cell wall which cleaves the amino acid L-alanine from various peptides. Significant activity is found almost only in Gram-negative microorganisms, all Gram-positive or Gram-variable microorganisms so far studied display no or very weak activity (Cerny 1976, Carlone et al. 1983). To perform the test, the reagent is used to make a suspension (with the bacteria). Aminopeptidase activity of the bacteria causes the release of 4-nitroaniline from the reagent, turning the suspension yellow. The test is especially useful for non-fermenters and gram-variable organisms, and is a one step test with several suppliers of kits. Results of the test are available in 5 minutes.

Fluorescent Stains

A popular combination of fluorescent stains for use in gram staining (particularly for flow-cytometry) involves the use of the fluorescent nucleic acid binding dyes hexidium iodide (HI) and SYTO 13. HI penetrates gram-positive but not gram-negative organisms, but SYTO 13 penetrates both. When the dyes were used together in a single step, gram-negative organisms are green fluorescent by SYTO 13 while gram-positive organisms are red-orange fluorescent by HI which overpowers the green of SYTO 13 (Mason et al 1998). There are commercial kits available for this procedure, which requires a fluorescent microscope or a flow cytometer.

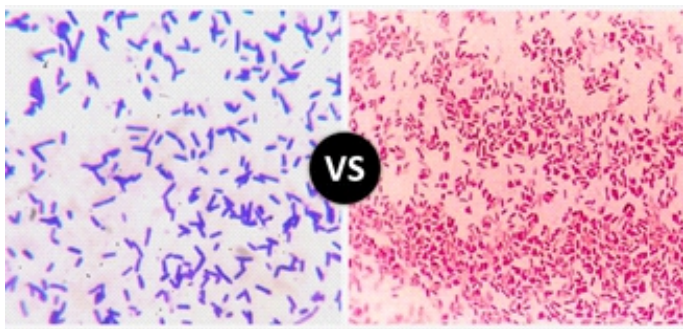
Sizemore et al (1990) developed a different approach to fluorescent labeling of cells. Fluorescence-labeled wheat germ agglutinin binds specifically to N-acetylglucosamine in the outer peptidoglycan layer of gram-positive bacteria. The peptidoglycan layer of gram-negative bacteria is covered by a membrane and is not labeled by the lectin. A variant of this method has also been used to “gram stain” microorganisms in milk for direct measurement by flow cytometry.

LAL-based Assay

Charles River Laboratories has just released a product to be used with their PTS instrument – the PTS Gram ID (Farmer 2005). This methodology makes use of the same reaction used for the chromogenic LAL test. Gram-negative organisms, with bacterial endotoxin, initiate the LAL coagulase cascade which results in activation of the proclotting enzyme, a protease. In the LAL test, this enzyme cleaves a peptide from the horseshoe crab coagulen, resulting in a clot. It can also cleave a peptide from a synthetic substrate, yielding a chromophore (p-nitroaniline) which is yellow and can be measured photometrically at 385 nm (Iwanaga 1987). Gram-positive organisms, lacking endotoxin, do not trigger the color change in this method, while gram-negative organisms do trigger it. Results are available within 10 minutes.

Summary

The differentiation of bacteria into either the gram-positive or the gram-negative group is fundamental to most bacterial identification systems. This task is usually accomplished through the use of Gram's Staining Method. Unfortunately, the gram stain methodology is complex and prone to error. This operator-dependence can be addressed by attention to detail, and by the use of controls on the test. Additional steps might include confirmatory tests, of which several examples were given. As with all microbiology assays, full technician training and competent review of the data are critical quality control steps for good laboratory results.



Gram Positive Bacteria vs. Gram Negative Bacteria

BOUQUET

In Lighter Vein

EXAM PATTERN
1997: Answer All the questions
2007: Answer any 5 questions
2017: Select Correct answers (A,B or C)
2022: Write Correct answer A or B
2027: Please only read the questions
2032: Thanks for Coming

We pronounce

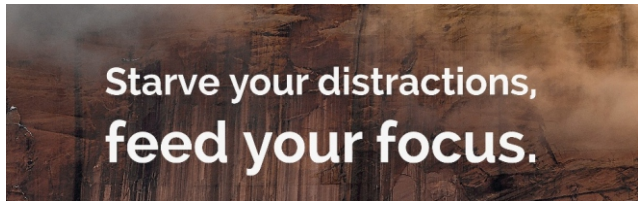
22 as Twenty Two
33 as Thirty Three
44 as Forty four
55 as Fifty five, Then why not say
11 as Onety One???
Doubt by Last Bench association.

Never ask Women about her age
Never ask men about his salary
Never Ask Student about his percentage

MY DREAM:

MY MOTHERS DREAM:

Wisdom Whispers



Brain Teasers

1. **What type of bacteria causes botulism?**
   A. Salmonella enterica
   B. Clostridium botulinum
   C. Escherichia coli
   D. Staphylococcus aureus

2. **How can botulinum toxin in food be destroyed?**
   A. Freezing
   B. Refrigeration
   C. Boiling for 10+ minutes
   D. Drying

3. **Which type of botulism is associated with drug injection or wound contamination?**
   A. Foodborne
   B. Infant
   C. Wound
   D. Inhalation

4. **Which of the following is a symptom of botulism?**
   A. High fever
   B. Skin rash
   C. Double vision or blurred vision
   D. Joint pain

ANSWER: 1-B, 2-C, 3-C, 4-C

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