VOLUME - XIV
ISSUE - LXXXIV
NOV/DEC 2017

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

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Editorial

Tetanus, also known as **lockjaw**, is an infection characterized by muscle spasms. In the most common type, the spasms begin in the jaw and then progress to the rest of the body. These spasms usually last a few minutes each time and occur frequently for three to four weeks. Spasms may be so severe that bone fractures may occur. Other symptoms may include fever, sweating, headache, trouble swallowing, high blood pressure, and a fast heart rate. Onset of symptoms is typically three to twenty-one days following infection. It may take months to recover. About 10% of those infected die.

Tetanus is caused by an infection with the bacterium *Clostridium tetani*, which is commonly found in soil, saliva, dust, and manure. The bacteria generally enter through a break in the skin such as a cut or puncture wound by a contaminated object. They produce toxins that interfere with muscle contractions, resulting in the typical symptoms. Diagnosis is based on the presenting signs and symptoms. The disease does not spread between people.

Infection can be prevented by proper immunization with the tetanus vaccine. In those who have a significant wound and less than three doses of the vaccine, both immunization and tetanus immune globulin are recommended. The wound should be cleaned and any dead tissue should be removed. In those who are infected tetanus immune globulin or, if it is not available, intravenous immunoglobulin (IVIG) is used. Muscle relaxants may be used to control spasms. Mechanical ventilation may be required if a person's breathing is affected.

Tetanus occurs in all parts of the world but is most frequent in hot and wet climates where the soil contains a lot of organic matter. In 2015 there were about 209,000 infections and about 59,000 deaths globally. This is down from 356,000 deaths in 1990. Description of the disease by Hippocrates exists from at least as far back as the 5th century BC. The cause of the disease was determined in 1884 by Antonio Carle and Giorgio Rattone at the University of Turin, with a vaccine being developed in 1924. Ourlead articlei n this issue **"DISEASE DIAGNOSIS"** aptly handles all aspects (clinico-diagnostic and therapeutic) as related to TETANUS.

When the primary segment talks about TETANUS, how can **"TROUBLE SHOOTING"** and **"INTERPRETATION"** talk about anything else except ANAEROBES and their culture/ isolation techniques. This makes it complete in all aspects.

Have we forgotten **"BOUQUET"**? Noooo……Just peep inside and flip over!

Let us know if you would like us to cover any other articles of your choice.

DISEASE DIAGNOSIS

TETANUS

Background

Tetanus is characterized by an acute onset of hypertonia, painful muscular contractions (usually of the muscles of the jaw and neck), and generalized muscle spasms without other apparent medical causes. Despite widespread immunization of infants and children since the 1940s, tetanus still occurs internationally.

Tetanus may be categorized into the following 4 clinical types:

- Generalized tetanus
- Localized tetanus
- Cephalic tetanus
- Neonatal tetanus

Approximately 50-75% of patients with generalized tetanus present with trismus ("lockjaw"), which is the inability to open the mouth secondary to masseter muscle spasm. Nuchal rigidity and dysphagia are also early complaints that cause risus sardonicus, the scornful smile of tetanus, resulting from facial muscle involvement. As the disease progresses, patients have generalized muscle rigidity with intermittent reflex spasms in response to stimuli (eg, noise, touch). Tonic contractions cause opisthotonos (ie, flexion and adduction of the arms, clenching of the fists, and extension of the lower extremities). During these episodes, patients have an intact sensorium and feel severe pain. The spasms can cause fractures, tendon ruptures, and acute respiratory failure. Patients with localized tetanus present with persistent rigidity in the muscle group close to the injury site. The muscular rigidity is caused by a dysfunction in the interneurons that inhibit the alpha motor neurons of the affected muscles. No further central nervous system (CNS) involvement occurs in this form, and mortality is very low. Cephalic tetanus is uncommon and usually occurs after head trauma or otitis media. Patients with this form present with cranial nerve (CN) palsies. The infection may be localized or may become generalized. Neonatal tetanus (tetanus neonatorum) is a major cause of infant mortality in underdeveloped countries but is rare in the advanced nations. Infection results from umbilical cord contamination during unsanitary delivery, coupled with a lack of maternal immunization. At the end of the first week of life, infected infants become irritable, feed poorly, and develop rigidity with spasms. Neonatal tetanus has a very poor prognosis. Although at present, tetanus is rare, it has not been eradicated, and early diagnosis and intervention are lifesaving. Prevention is the ultimate management strategy for tetanus.

Pathophysiology

Clostridium tetani is an obligate, anaerobic, motile, gram-positive bacillus. It is nonencapsulated and forms spores that are resistant to heat, desiccation, and disinfectants. Since the colorless spores are located at one end of the bacillus, they cause the organism to resemble a turkey leg. They are found in soil, house dust, animal intestines, and human feces. Spores can persist in normal tissue for months to years. To germinate, the spores require specific anaerobic conditions, [6] such as wounds with low oxidation-reduction potential (eg, dead or devitalized tissue, foreign body, active infection). Under these conditions, upon germination, they may release their toxin. Infection by *C tetani* results in a benign appearance at the portal of entry because of the inability of the organism to evoke an inflammatory reaction unless coinfection with other organisms develops. When the proper anaerobic conditions are present, the spores germinate and produce the following 2 toxins:

- Tetanolysin – This substance is a hemolysin with no recognized pathologic activity
- Tetanospasmin – This toxin is responsible for the clinical manifestations of tetanus [7]; by weight, it is one of the most potent toxins known, with an estimated minimum lethal dose of 2.5 ng/kg body weight

Tetanospasmin is synthesized as a 150-kd protein consisting of a 100-kd heavy chain and a 50-kd light chain joined by a disulfide bond. The heavy chain mediates binding of tetanospasmin to the presynaptic motor neuron and also creates a pore for the entry of the light chain into the cytosol. The light chain is a zinc-dependent protease that cleaves synaptobrevin. After the light chain enters the motor neuron, it travels by retrograde axonal transport from the contaminated site to the spinal cord in 2-14 days. When the toxin reaches the spinal cord, it enters central inhibitory neurons. The light chain cleaves the protein synaptobrevin, which is integral to the binding of neurotransmitter containing vesicles to the cell membrane. As a result, gamma-aminobutyric acid (GABA)-containing and glycine-containing vesicles are not released, and there is a loss of inhibitory action on motor and autonomic neurons. [9] With this loss of central inhibition, there is autonomic hyperactivity as well as uncontrolled muscle contractions (spasms) in response to normal stimuli such as noises or lights. Once the toxin becomes fixed to neurons, it cannot be neutralized with antitoxin. Recovery of nerve function from tetanus toxins requires sprouting of new nerve terminals and formation of new synapses. Localized tetanus develops when only the nerves supplying the affected muscle are involved. Generalized tetanus develops when the toxin released at the wound spreads through the lymphatics and blood to multiple nerve terminals. The blood-brain barrier prevents direct entry of toxin to the CNS.

Etiology

Tetanus spores may survive for years in some environments and are resistant to disinfectants and to boiling for 20 minutes. However, vegetative cells are easily inactivated and are susceptible to several antibiotics. Tetanus can be acquired outdoors as well as indoors. The source of infection usually is a wound (approximately 65% of cases), which often is minor (eg, from wood or metal splinters or thorns). Frequently, no initial medical treatment is sought. Chronic skin ulcers are the source in approximately 5% of cases. In the remainder of cases, no obvious source can be identified. Tetanus can also develop as a complication of chronic conditions such as abscesses and gangrene. It may infect tissue damaged by burns, frostbite, middle ear infections, dental or surgical procedures, abortion, childbirth, and intravenous (IV) or subcutaneous drug use. In addition, possible sources not usually associated with tetanus include intranasal and other foreign bodies and corneal abrasions. Underimmunization is an important cause of tetanus. Tetanus affects nonimmunized persons, partially immunized persons, or fully immunized individuals who do not maintain adequate immunity with periodic booster doses. Only 12-14% of patients with tetanus in the United States have received a primary series of tetanus toxoid. During 1998-2000, only 6% of all patients with tetanus were known to be current with tetanus immunization, with no fatal cases reported among this group. Surveillance data from this period revealed the following:

- In 73% of patients with tetanus in the advanced countries tetanus occurred after an acute injury, including puncture wounds (50%), lacerations (33%), and abrasions (9%)
- Stepping on a nail accounted for 32% of the puncture wounds
- Tetanus was found to occur in burn victims; in patients receiving intramuscular injections; in persons obtaining a tattoo; and in

persons with frostbite, dental infections (eg, periodontal abscesses), penetrating eye injuries, and umbilical stump infections
- Other reported risk factors included diabetes, chronic wounds (eg, skin ulcers, abscesses, or gangrene), parenteral drug abuse, and recent surgery (4% of US cases)
- During 1998-2000, 12% of patients with tetanus in the United States had diabetes (with mortality, 31%), compared with 2% during 1995-1997; of these patients, 69% had acute injuries and 25% had gangrene or a diabetic ulcer
- The median time interval between surgery and onset of tetanus was 7 days
- Tetanus was reported after tooth extractions, root canal therapy, and intraoral soft tissue trauma

Worldwide risk factors for neonatal tetanus include the following:
- Unvaccinated mother, home delivery, and unhygienic cutting of the umbilical cord increase susceptibility to tetanus
- A history of neonatal tetanus in a previous child is a risk factor for subsequent neonatal tetanus
- Potentially infectious substances applied to the umbilical stump (eg, animal dung, mud, or clarified butter) are risk factors for neonates

Immunity from tetanus decreases with advancing age. Serologic testing for immunity has revealed a low level among elderly individuals in the United States. Approximately 50% of adults older than 50 years are nonimmune because they never were vaccinated or do not receive appropriate booster doses. The prevalence of immunity to tetanus in the United States exceeds 80% for persons aged 6-39 years but is only 28% for those older than 70 years.

EPIDEMIOLOGY

International statistics

C tetani is found worldwide in soil, on inanimate objects, in animal feces, and, occasionally, in human feces. Tetanus is predominantly a disease of underdeveloped countries. It is common in areas where soil is cultivated, in rural areas, in warm climates, during summer months, and among males. In countries without a comprehensive immunization program, tetanus predominantly develops in neonates and young children. Developed nations have incidences of tetanus similar to those observed in the United States. For instance, only 126 cases of tetanus were reported in England and Wales from 1984-1992. Although tetanus affects all ages, the highest prevalence is in newborns and young people. In 1992, an estimated 578,000 infant deaths were attributed to neonatal tetanus. In 1998, 215,000 deaths occurred, more than 50% of them in Africa. Tetanus is a target disease of the World Health Organization (WHO) Expanded Program on Immunization. Overall, the annual incidence of tetanus is 0.5-1 million cases. WHO estimated that in 2002, there were 213,000 tetanus deaths, 198,000 of them in children younger than 5 years.

Age-related demographics

Neonatal tetanus is rare, occurring most frequently in countries without comprehensive vaccination programs. The risk for development of tetanus and for the most severe form of the disease is highest in the elderly population. In the advanced nations, 59% of cases and 75% of deaths occur in persons aged 60 years or older. From 1980 through 2000, 70% of reported cases of tetanus in the developed countries were among persons aged 40 years or older. Of all these patients, 36% are older than 59 years and only 9% are younger than 20 years.

Sex-related demographics

Tetanus affects both sexes. No overall gender predilection has been reported, except to the extent that males may have more soil exposure in some cultures. In the United States from 1998 to 2000, the incidence of tetanus was 2.8 times higher in males aged 59 years and younger than in females in the same age range. A difference in the levels of tetanus immunity exists between the sexes. Overall, men are believed to be better protected than women, perhaps because of additional vaccinations administered during military service or professional activities. In developing countries, women have an increased immunity where tetanus toxoid is administered to women of childbearing age to prevent neonatal tetanus.

Race-related demographics

Tetanus affects all races.

Prognosis

The prognosis is dependent on incubation period, the time from spore inoculation to first symptom, and the time from first symptom to first tetanic spasm. The following statements typically hold true:
- In general, shorter intervals indicate more severe tetanus and a poorer prognosis
- Patients usually survive tetanus and return to their predisease state of health
- Recovery is slow and usually occurs over 2-4 months
- Some patients remain hypotonic
- Clinical tetanus does not produce a state of immunity; therefore, patients who survive the disease require active immunization with tetanus toxoid to prevent a recurrence

A rating scale has been developed for assessing the severity of tetanus and determining the prognosis. On this scale, 1 point is given for each of the following:
- Incubation period shorter than 7 days
- Period of onset shorter than 48 hours
- Tetanus acquired from burns, surgical wounds, compound fractures, septic abortion, umbilical stump, or intramuscular injection
- Narcotic addiction
- Generalized tetanus
- Temperature higher than 104°F (40°C)
- Tachycardia exceeding 120 beats/min (150 beats/min in neonates)

The total score indicates disease severity and prognosis as follows:
- 0 or 1 – Mild tetanus; mortality below 10%
- 2 or 3 – Moderate tetanus; mortality of 10-20%
- 4 – Severe tetanus; mortality of 20-40%
- 5 or 6 – Very severe tetanus; mortality above 50%

Cephalic tetanus is always severe or very severe. Neonatal tetanus is always very severe. Current statistics indicate that mortality in mild and moderate tetanus is approximately 6%; for severe tetanus, it may be as high as 60%. Mortality in the advanced countries resulting from generalized tetanus is 30% overall, 52% in patients older than 60 years, and 13% in patients younger than 60 years. Mortality is substantially higher for people older than 60 years (40%) than for those aged 20-59 years (8%). From 1998 to 2000, 75% of the deaths in the United States were in patients older than 60 years. In addition, mortality is notably higher for people who require mechanical ventilation (30%) than for those who do not (4%).

A high risk of mortality is associated with the following:
- Short incubation period
- Early onset of convulsions
- Delay in treatment
- Contaminated lesions of the head and the face
- Neonatal tetanus

Clinical tetanus is less severe among patients who have received a primary series of tetanus toxoid sometime during their life than among

patients who are inadequately vaccinated or unvaccinated. Mortality in the United States is 6% for individuals who had previously received 1-2 doses of tetanus toxoid, compared with 15% for individuals who were unvaccinated. Residual neurologic sequelae are uncommon. Mortality usually results from autonomic dysfunction (eg, extremes in blood pressure, dysrhythmias, or cardiac arrest).

Patient Education

The importance of childhood immunizations and boosters must be stressed. Midwives and birth attendants in developing and underdeveloped countries should be given training in aseptic birthing procedures. The basics of wound care and first aid should be widely taught. Early recognition of symptoms and signs of localized tetanus and timely access to medical care are essential.

Tetanus Clinical Presentation

History

Most cases of tetanus in the United States occur in patients with a history of underimmunization, either because they were never vaccinated or because they completed a primary series but have not had a booster in the preceding 10 years. From 1995 to 1997, 54% of the reported cases in the United States had an unknown tetanus vaccination history, 22% had no known previous tetanus vaccination, 9% had 1 previous dose, 3% had 2, 3% had 3, and 9% had 4 or more. Persons who inject drugs also constitute a high-risk group. The median incubation period is 7 days, and for most cases (73%), incubation ranges from 4 to 14 days. The incubation period is shorter than 4 days in 15% of cases and longer than 14 days in 12% of cases. Patients with clinical manifestations occurring within 1 week of an injury have more severe clinical courses. Patients sometimes remember an injury, but often, the injury goes unnoticed. Patients may report a sore throat with dysphagia (early sign). The initial manifestation may be local tetanus, in which the rigidity affects only 1 limb or area of the body where the clostridium-containing wound is located. Patients with generalized tetanus present with trismus (ie, lockjaw) in 75% of cases. Other presenting complaints include stiffness, neck rigidity, restlessness, and reflex spasms. Subsequently, muscle rigidity becomes the major manifestation. Muscle rigidity spreads in a descending pattern from the jaw and facial muscles over the next 24-48 hours to the extensor muscles of the limbs. Dysphagia occurs in moderately severe tetanus as a consequence of pharyngeal muscle spasms, and onset is usually insidious over several days. Reflex spasms develop in most patients and can be triggered by minimal external stimuli such as noise, light, or touch. The spasms last seconds to minutes; become more intense; increase in frequency with disease progression; and can cause apnea, fractures, dislocations, and rhabdomyolysis. Laryngeal spasms can occur at any time and can result in asphyxia. Other symptoms include elevated temperature, sweating, elevated blood pressure, and episodic rapid heart rate. Sustained contraction of facial musculature produces a sneering grin expression known as risus sardonicus.

Generalized tetanus

Generalized tetanus is the most commonly found form of tetanus in the United States, accounting for 85-90% of cases. The extent of the trauma varies from trivial injury to contaminated crush injury. The incubation period is 7-21 days, largely depending on the distance of the injury site from the central nervous system (CNS). Trismus is the presenting symptom in 75% of cases; a dentist or an oral surgeon often initially sees the patient. Other early features include irritability, restlessness, diaphoresis, and dysphagia with hydrophobia, drooling, and spasm of the back muscles. These early manifestations reflect involvement of bulbar and paraspinal muscles, possibly because these structures are innervated by the shortest axons. The condition may progress for 2 weeks despite antitoxin therapy because of the time needed for intra-axonal antitoxin transport.

Localized tetanus

Localized tetanus involves an extremity with a contaminated wound and is of highly variable severity. It is an unusual form of tetanus, and the prognosis for survival is excellent.

Cephalic tetanus

Cephalic tetanus generally follows head injury or develops with infection of the middle ear. Symptoms consist of isolated or combined dysfunction of the cranial motor nerves (most frequently CN VII). Cephalic tetanus may remain localized or may progress to generalized tetanus. It is an unusual form of tetanus with an incubation period of 1-2 days. The prognosis for survival is usually poor.

Physical Examination

Common first signs of tetanus are headache and muscular stiffness in the jaw (ie, lockjaw), followed by neck stiffness, difficulty swallowing, rigidity of abdominal muscles, spasms, and sweating. Patients often are afebrile. Stimulation of the posterior pharyngeal wall may elicit reflex spasms of the masseter muscles that cause patients to bite down as opposed to gag (spatula test).[17] Severe tetanus results in opisthotonos, flexion of the arms, extension of the legs, periods of apnea resulting from spasm of the intercostal muscles and diaphragm, and rigidity of the abdominal wall. Late in the disease, autonomic dysfunction develops, with hypertension and tachycardia alternating with hypotension and bradycardia; cardiac arrest may occur. The lower extremity is the site of antecedent acute injury in 52% of patients, the upper extremity is the site of antecedent injury in 34% of patients, and the head or the trunk is the site of antecedent injury in 5% of patients. Tetanic seizures may occur. Their presence portends a poor prognosis, and their frequency and severity are related to the severity of the disease. These seizures resemble epileptic seizures, with the presence of a sudden burst of tonic contractions. However, the patient does not lose consciousness and usually experiences severe pain. Seizures frequently occur in the muscle groups causing opisthotonos, flexion and abduction of the arms, clenching of the fists against the thorax, and extension of the lower extremities. Patients with tetanus may present with abdominal tenderness and guarding, mimicking an acute abdomen. Exploratory laparotomies have been performed before the correct diagnosis was apparent.

Tetanospasmin has a disinhibitory effect on the autonomic nervous system (ANS). ANS dysfunction becomes progressively evident as the level of toxin in the CNS increases. ANS disturbances (eg, sweating, fluctuating blood pressure, episodic tachydysrhythmia, and increased catecholamine release) are observed. Drugs with beta-blocker effects have been used to control the cardiovascular manifestations of ANS instability, but they also have been associated with increased risk of sudden death.

Generalized tetanus

Sustained trismus may result in the characteristic sardonic smile (risus sardonicus) and persistent spasm of the back musculature may cause opisthotonos. Waves of opisthotonos are highly characteristic of the disease. With progression, the extremities become involved in episodes of painful flexion and adduction of the arms, clenched fists, and extension of the legs. Noise or tactile stimuli may precipitate spasms and generalized convulsions. Involvement of the ANS may result in severe arrhythmias, oscillation of blood pressure, profound diaphoresis, hyperthermia, rhabdomyolysis, laryngeal spasm, and urinary retention.

In most cases, the patient remains lucid.

Localized tetanus

In mild cases of localized tetanus, patients may have weakness of the involved extremity, presumably due to partial immunity; in more severe cases, they may have intense, painful spasms of the group of muscles in close proximity to the site of injury. This disorder may persist for several weeks but is usually self-limiting; however, more severe cases tend to progress to generalized tetanus.

Cephalic tetanus

Cephalic tetanus is a rare form of the disease that is usually secondary to chronic otitis media or head trauma. It is characterized by variable CN palsies, most frequently involving CN VII. Ophthalmoplegic tetanus is a variant that develops after penetrating eye injuries and results in CN III palsies and ptosis. Rapid progression is typical. Cephalic tetanus may remain localized or, especially if left untreated, progress to generalized tetanus.

Neonatal tetanus

Neonatal tetanus presents with an inability to suck 3-10 days after birth. Presenting symptoms include irritability, excessive crying, grimaces, intense rigidity, and opisthotonos. In general, the physical examination findings are similar to those of generalized tetanus.

Neonatal tetanus

Neonatal tetanus (tetanus neonatorum) is generalized tetanus that results from infection of a neonate. It primarily occurs in underdeveloped countries and accounts for as many as one half of all neonatal deaths. The usual cause is the use of contaminated materials to sever or dress the umbilical cord in newborns of unimmunized mothers. The usual incubation period after birth is 3-10 days, which explains why this form of tetanus is sometimes referred to as the disease of the seventh day. The newborn usually exhibits irritability, poor feeding, rigidity, facial grimacing, and severe spasms with touch. Mortality exceeds 70%.

Complications

Complications include spasm of the vocal cords and spasm of the respiratory muscles that cause interference with breathing. [18] Patients experience severe pain during each spasm. During the spasm, the upper airway can be obstructed, or the diaphragm may participate in the general muscular contraction. Sympathetic overactivity is the major cause of tetanus-related death in the intensive care unit (ICU). Sympathetic hyperactivity usually is treated with labetalol at 0.25-1 mg/min as needed for blood pressure control or with morphine at 0.5-1 mg/kg/h by continuous infusion. Neonatal tetanus follows infection of the umbilical stump, most commonly resulting from a failed aseptic technique in a mother who is inadequately immunized. Mortality for neonatal tetanus exceeds 90%, and developmental delays are common among survivors. Before 1954, asphyxia from tetanic spasms was the usual cause of death in patients with tetanus. However, with the advent of neuromuscular blockers, mechanical ventilation, and pharmacologic control of spasms, sudden cardiac death has become the leading cause of death. Sudden cardiac death has been attributed to excessive catecholamine productions or the direct action of tetanospasmin or tetanolysin on the myocardium. Nosocomial infections are common when hospitalization is prolonged. Secondary infections may include sepsis from decubitus ulcers, hospital-acquired pneumonia, and catheter-related infections. Pulmonary embolism is a particular problem in drug users and elderly patients.

Further complications include the following:

- Long bone fractures
- Glenohumeral joint and temporomandibular joint dislocations
- Hypoxic injury and aspiration pneumonia
- Clotting in the blood vessels of the lung
- Adverse effects of autonomic instability,[19] including hypertension and cardiac dysrhythmias
- Paralytic ileus, pressure sores, and urinary retention
- Malnutrition and stress ulcers
- Coma, nerve palsies, neuropathies, psychological aftereffects, and flexion contractures

Tetanus Differential Diagnoses

Diagnostic Considerations

Strychnine poisoning is the only condition that truly mimics tetanus. However, a number of conditions (eg, dental or other local infections, hysteria, neoplasms, and encephalitis) may cause trismus, and these must be differentiated these conditions from tetanus. The following conditions listed do not cause manifestations of tetanus other than trismus:

- Strychnine poisoning
- Dental infections
- Local infections
- Hysteria
- Neoplasms
- Malignant hyperthermia
- Stimulant use
- Intraoral disease
- Odontogenic infections
- Globus hystericus
- Hepatic encephalopathy
- Acute abdomen
- Intracranial hemorrhage
- Dystonic drug reactions (eg, phenothiazines, metoclopramide)
- Acute abdominal emergencies
- Seizure disorder (partial or generalized)
- Serotonin syndrome
- Stroke, ischemic (cephalic tetanus)

Differential Diagnoses

- Arthrogryposis
- Conversion Disorder in Emergency Medicine
- Emergency Treatment of Rabies
- Emergent Management of Subarachnoid Hemorrhage
- Encephalitis
- Hemorrhagic Stroke
- Mandible Dislocation
- Medication-Induced Dystonic Reactions
- Neuroleptic Malignant Syndrome
- Pediatric Aseptic Meningitis
- Pediatric Bacterial Meningitis
- Peritonsillar Abscess in Emergency Medicine
- Tardive Dystonia
- Widow Spider Envenomation

Tetanus Workup

Laboratory Studies

No specific laboratory tests exist for determining the diagnosis of tetanus. The diagnosis is clinically based on the presence of trismus, dysphagia, generalized muscular rigidity, spasm, or combinations thereof. Although the laboratory findings are not diagnostically valuable, they may help exclude strychnine poisoning. Blood counts and blood chemical findings are unremarkable. Laboratory studies may

demonstrate a moderate peripheral leukocytosis. A lumbar puncture is not necessary for diagnosis. Cerebrospinal fluid (CSF) findings are normal, except for an increased opening pressure, especially during spasms. Serum muscle enzyme levels (eg, creatine kinase, aldolase) may be elevated. An assay for antitoxin levels is not readily available. However, a serum antitoxin level of 0.01 IU/mL or higher is generally considered protective, making the diagnosis of tetanus less likely (though rare cases have been reported to occur despite the presence of protective antitoxin levels). Wounds should be cultured in cases of suspected tetanus. It must be kept in mind, however, that *C tetani* sometimes can be cultured from the wounds of patients who do not have tetanus and frequently cannot be cultured from the wounds of patients who do.

Spatula Test

The spatula test is a simple diagnostic bedside test that involves touching the oropharynx with a spatula or tongue blade. In normal circumstances, it elicits a gag reflex, and the patient tries to expel the spatula (ie, a negative test result). If tetanus is present, patients develop a reflex spasm of the masseters and bite the spatula (ie, a positive test result). In 400 patients, this test had a sensitivity of 94% and a specificity of 100%.[17] No adverse sequelae (eg, laryngeal spasm) were reported.

Other Studies

Electromyography (EMG) may show continuous discharge of motor subunits and shortening or absence of the silent interval normally observed after an action potential. Nonspecific changes may be evident on electrocardiography (ECG). Imaging studies of the head and spine reveal no abnormalities.

Tetanus Treatment & Management

Approach Considerations

The goals of treatment in patients with tetanus include the following:

- Initiating supportive therapy
- Debriding the wound to eradicate spores and alter conditions for germination
- Stopping the production of toxin within the wound
- Neutralizing unbound toxin
- Controlling disease manifestations
- Managing complications

Patients should be admitted to an intensive care unit (ICU). If the facility does not have an ICU, the patient should be transferred by critical care ambulance. Passive immunization with human tetanus immune globulin (TIG) shortens the course of tetanus and may lessen its severity. A dose of 500 U may be as effective as larger doses. Therapeutic TIG (3,000-6,000 units as 1 dose) has also been recommended for generalized tetanus. Other treatment measures include ventilatory support, high-calorie nutritional support, and pharmacologic agents that treat reflex muscle spasms, rigidity, tetanic seizures and infections.

Initial Supportive Therapy and Wound Care

Patients should be admitted to the ICU. Because of the risk of reflex spasms, a dark and quiet environment should be maintained. Unnecessary procedures and manipulations should be avoided. Prophylactic intubation should be seriously considered in all patients with moderate-to-severe clinical manifestations. Intubation and ventilation are required in 67% of patients. Attempting endotracheal intubation may induce severe reflex laryngospasm; preparations must be made for emergency surgical airway control. Rapid sequence intubation techniques (eg, with succinylcholine) are recommended to avoid this complication. Tracheostomy should be performed in patients requiring intubation for more than 10 days. Tracheostomy has also been recommended after onset of the first generalized seizure. The possibility of developing tetanus directly correlates with the characteristics of the wound. Recently acquired wounds with sharp edges that are well vascularized and not contaminated are least likely to develop tetanus. All other wounds are considered predisposed to tetanus. The most susceptible wounds are those that are grossly contaminated or that are caused by blunt trauma or bites. Wounds should be explored, carefully cleansed, and properly debrided. In many cases, the wound responsible for tetanus is clear at presentation, in which case surgical debridement offers no significant benefit. If debridement is indicated, it should be undertaken only after the patient has been stabilized. The current recommendation is to excise at least 2 cm of normal viable-appearing tissue around the wound margins. Abscesses should be incised and drained. Because of the risk of releasing tetanospasmin into the bloodstream, any wound manipulation should be delayed until several hours after administration of antitoxin.

Pharmacologic Therapy

Elimination of toxin production

Antimicrobials are used to decrease the number of vegetative forms of *C tetani* (the toxin source) in the wound. For years, penicillin G was used widely for this purpose, but it is not the current drug of choice. Metronidazole (eg, 0.5 g every 6 hours) has comparable or better antimicrobial activity, and penicillin is a known antagonist of gamma-aminobutyric acid (GABA), as is tetanus toxin. Metronidazole is also associated with lower mortality. Other antimicrobials that have been used are clindamycin, erythromycin, tetracycline, and vancomycin. Their role is not well established.

Neutralization of unbound toxin

Tetanus immune globulin (TIG) is recommended for treatment of tetanus. It should be kept in mind that TIG can only help remove unbound tetanus toxin; it cannot affect toxin bound to nerve endings. A single intramuscular (IM) dose of 3000-5000 units is generally recommended for children and adults, with part of the dose infiltrated around the wound if it can be identified. The World Health Organization recommends TIG 500 units by IM injection or intravenously (IV)—depending on the available preparation—as soon as possible; in addition, 0.5 mL of an age-appropriate tetanus toxoid- containing vaccine (Td, Tdap, DT, DPT, DTaP, or tetanus toxoid, depending on age or allergies), should be administered by IM injection at a separate site. Tetanus disease does not induce immunity; patients without a history of primary tetanus toxoid vaccination should receive a second dose 1-2 months after the first dose and a third dose 6-12 months later.

Control of disease manifestations

Benzodiazepines have emerged as the mainstay of symptomatic therapy for tetanus. Diazepam is the most frequently studied and used drug; it reduces anxiety, produces sedation, and relaxes muscles. Lorazepam is an effective alternative. High dosages of either may be required (up to 600 mg/day). To prevent spasms that last longer than 5-10 seconds, administer diazepam IV, typically 10-40 mg every 1-8 hours. Vecuronium (by continuous infusion) or pancuronium (by intermittent injection) are adequate alternatives. Midazolam 5-15 mg/hr IV has been used. If the spasms are not controlled with benzodiazepines, long-term neuromuscular blockade is required. Phenobarbital is another anticonvulsant that may be used to prolong the effects of diazepam. Phenobarbital is also used to treat severe muscle spasms and provide sedation when neuromuscular blocking agents are used. Other agents used for spasm control include baclofen, dantrolene, short-acting barbiturates, and chlorpromazine. Propofol has been suggested for

sedation. Intrathecal (IT) baclofen, a centrally acting muscle relaxant, has been used experimentally to wean patients off the ventilator and to stop diazepam infusion. IT baclofen is 600 times more potent than oral baclofen. Repeated IT injections have been efficacious in limiting duration of artificial ventilation or preventing intubation. Case reports and small case series have suggested that IT baclofen is effective in controlling muscle rigidity, though others have questioned this. The effects of baclofen begin within 1-2 hours and persist for 12-48 hours. The half-life elimination of baclofen in cerebrospinal fluid (CSF) ranges from 0.9 to 5 hours. After lumbar IT administration, the cervical-to-lumbar concentration ratio is 1:4. The major adverse effect is a depressed level of consciousness and respiratory compromise.

Management of complications

Specific therapy for autonomic system complications and control of spasms should be initiated.[26] Magnesium sulfate can be used alone or in combination with benzodiazepines for this purpose. It should be given IV in a loading dose of 5 g (or 75 mg/kg), followed by continuous infusion at a rate of 2-3 g/h until spasm control is achieved.[7] The patellar reflex should be monitored; areflexia (absence of the patellar reflex) occurs at the upper end of the therapeutic range (4 mmol/L). If areflexia develops, the dosage should be reduced. An infusion of magnesium sulfate does not reduce the need for mechanical ventilation in adults with severe tetanus, but it does reduce the requirement for other drugs to control muscle spasms and cardiovascular instability.[27] In a meta-analysis of 3 controlled trials that compared magnesium sulfate with placebo or diazepam for the treatment of patients with tetanus, magnesium sulfate did not reduce mortality or relative risk.[28] The investigators concluded that further controlled trials were necessary to evaluate the potential effect of this therapy on autonomic dysfunction, spasms, length of ICU and hospital stay, and requirement for mechanical ventilation. Morphine is an option. In the past, beta blockers were used, but they can cause hypotension and sudden death; only esmolol is currently recommended. Hypotension requires fluid replacement and dopamine or norepinephrine administration. Parasympathetic overactivity is rare, but if bradycardia is sustained, a pacemaker may be needed. Clinical tetanus does not induce immunity against future attacks; therefore, all patients should be fully immunized with tetanus toxoid during the convalescent period.

Diet and Activity

Maintenance of adequate nutrition is extremely important. Because of the risk of aspiration, patients should not be given any food by mouth. Nutrition should be provided to seriously ill patients via nasoduodenal tubes, gastrostomy tube feedings, or parenteral hyperalimentation. Consultation with a nutritionist is helpful. The patient should be on bed rest in a room that can be kept dark and quiet. Even the slightest physical stimulus can cause a cycle of spasms.

Consultations

An intensive care medicine specialist should be the primary physician coordinating the patient's care. Consultations with the following specialists may be appropriate as the clinical situation dictates:

- Infectious diseases
- Toxicology - To help confirm or exclude strychnine toxicity as the cause of symptoms
- Neurology - To confirm or exclude seizures as a possible etiology of symptoms
- Pulmonary medicine – To be consulted after admission to the ICU for patients with severe respiratory symptoms or those requiring mechanical ventilation
- Anesthesiology – To be consulted after admission to the ICU if intrathecal baclofen is to be administered

Prevention

Prevention of tetanus is accomplished through vaccination with DTP or DTaP at the ages of 2 months, 4 months, 6 months, 12-18 months, and 4-6 years. In 2006, the Advisory Committee on Immunization Practices (ACIP) issued recommendations for the use of Tdap. For persons aged 7 years or older who have never been vaccinated against tetanus, diphtheria, or pertussis (ie, have never received any dose of DTP/DTaP/DT or Td), administer a series of 3 vaccinations containing tetanus and diphtheria toxoids. The preferred schedule is a single dose of Tdap, followed by a dose of Td at least 4 weeks after Tdap and another dose of Td 6-12 months later. However, Tdap can be given once as a substitute for Td in the 3-dose primary series.[29] Alternatively, in situations where the adult probably received vaccination against tetanus and diphtheria but cannot produce a record, vaccine providers may consider serologic testing for antibodies to tetanus and diphtheria toxin with the aim of avoiding unnecessary vaccination. If tetanus and diphtheria antitoxin levels are each higher than 0.1 IU/mL, previous vaccination with tetanus and diphtheria toxoid vaccine is presumed, and a single dose of Tdap is indicated.[29] Adults who received other incomplete vaccination series against tetanus and diphtheria should be vaccinated with Td to complete a 3-dose primary series of tetanus and diphtheria toxoid-containing vaccines. One dose of Tdap should be used in place of Td if the patient has never received a dose of Tdap. Pregnancy is not a contraindication to the use of Tdap in the second and third trimester. Secondary prevention of tetanus is accomplished after exposure through appropriate wound cleansing and debridement and the administration of tetanus toxoid (Td, Tdap, DT, DPT, or DTaP, as indicated) and TIG, when indicated. Pediatric formulations (DT and DTaP) include about the same amount of tetanus toxoid as adult Td does but contain 3-4 times as much diphtheria toxoid.

The following wounds should be considered prone to tetanus:

- Wounds that have been present for longer than 6 hours
- Deep (>1 cm) wounds
- Grossly contaminated wounds
- Wounds that are exposed to saliva or feces, stellate, or ischemic or infected (including abscesses
- Avulsions, punctures, or crush injuries

It is not necessary to wait the typical 10 years to get the adult Tdap dose after the last Td dose. An interval as short as 2 years is suggested to reduce the likelihood of increased reactogenicity, and even shorter intervals may be appropriate if the patient is at high risk for pertussis, has close contact with infants, or may not be able to receive another vaccination. Providers should know that shorter intervals are not contraindicated, that accumulating data reinforce safety of the vaccine, and that there are no concerns about immunogenicity with the decreased interval. Patients with tetanus-prone wounds should receive Td or DPT IM if they are younger than 7 years and if it has been more than 5 years since their last dose of tetanus toxoid. Patients who have previously received fewer than 3 doses of tetanus toxoid and patients aged 60 years or older should receive TIG 250-500 units IM, always in the opposite extremity to the toxoid. Adults without tetanus-prone wounds should be given Td or Tdap if they have previously have received fewer than 3 doses of tetanus toxoid or if more than 10 years have passed since their last dose. Tdap is preferred to Td for adults vaccinated more than 5 years earlier who require tetanus toxoid as part of wound management and who have not previously received Tdap.

Tdap is indicated only once; therefore, for adults previously vaccinated with Tdap (after age 7 years), Td should be used if a tetanus toxoid- containing vaccine is indicated for wound care. It is important to review the immunization status of all patients who present to an emergency department for any care (regardless of chief complaint). Immunizations should be administered if a lapse of more than 10 years has occurred since the last tetanus booster. If a patient does not remember or cannot give a history of immunization, an immunochromatographic dipstick test may be appropriate and cost-effective for determining tetanus immunity in this setting, though further study is needed to determine the applicability of this approach. [30] The ACIP recommends vaccination at primary care visits for adolescents aged 11-12 years and for adults aged 50 years, review of vaccination histories, and updating of tetanus vaccination status. This is in addition to recommending booster doses of tetanus and diphtheria toxoid every 10 years.

In 2011 and 2012, the ACIP issued updated recommendations for the use of Tdap. Key points included the following:

- Timing of Tdap after Td – Pertussis vaccination, when indicated, should not be delayed; Tdap should be administered regardless of the interval since the last tetanus- or diphtheria toxoid- containing vaccine
- Tdap use in adults – All adults aged 19 years and older who have not yet received a dose of Tdap should receive a single dose, regardless of the interval since the last tetanus- or diphtheria toxoid- containing vaccine, then should continue to receive Td for routine booster immunization
- Wound management for adults – A tetanus toxoid–containing vaccine may be recommended as part of standard wound management in adults aged 19 years and older if it has been at least 5 years since last receipt of Td; if a tetanus booster is indicated, Tdap is preferred to Td for wound management in adults aged 19 years and older who have not received Tdap previously
- Adults aged 65 years and older – Those who have or anticipate having close contact with an infant younger than 12 months and have not received Tdap should receive a single dose of Tdap; others may be given a single dose of Tdap instead of Td if they have not previously received Tdap; Tdap can be administered regardless of the interval since the last tetanus- or diphtheria toxoid- containing vaccine; Td is then given for routine booster immunization
- When feasible, Boostrix should be used for adults aged 65 years and older; however, either of the 2 available vaccines administered to a person 65 years or older is immunogenic and would provide protection, and a dose of either may be considered valid
- Pregnant women who have not previously received Tdap – Tdap should be administered during pregnancy, preferably during the third or late second trimester (after 20 weeks' gestation); if not given during pregnancy, it should be given immediately post partum; if a booster vaccination is indicated during pregnancy, it should be given according to the same time frame
- Pregnant women with unknown or incomplete tetanus vaccination – To ensure protection, 3 vaccinations containing tetanus and reduced diphtheria toxoids should be given, ideally at 0 weeks, 4 weeks, and 6-12 months; Tdap should replace 1 dose of Td, preferably during the third or late second trimester
- Undervaccinated children aged 7-10 years – If there is no contraindication to pertussis vaccine, a single dose of Tdap is indicated; if additional doses of tetanus and diphtheria toxoid--containing vaccines are needed, vaccinated should proceed according to catch-up guidance, with Tdap preferred as the first dose

Worldwide, neonatal tetanus may be eliminated by increasing immunizations in women of childbearing age, especially pregnant women, and by improving maternity care. Administration of tetanus toxoid twice during pregnancy (4-6 weeks apart, preferably in the last 2 trimesters) and again at least 4 weeks before delivery is recommended for previously unimmunized gravid women. Maternal antitetanus antibodies are passed to the fetus, and this passive immunity is effective for many months.

MEDICATION

Medication Summary

The goals of pharmacotherapy are to stop toxin production within the wound, to neutralize unbound toxin, and to control disease manifestations. Drugs used to treat muscle spasm, rigidity, and tetanic seizures include sedative-hypnotic agents, general anesthetics, centrally acting muscle relaxants, and neuromuscular blocking agents. Antibiotics are used to prevent multiplication of *Clostridium tetani,* thus halting production and release of toxins. Antitoxins are given to neutralize unbound toxin.

Vaccines, Inactivated, Bacterial

Class Summary

Active immunization increases resistance to infection. Vaccines consist of microorganisms or cellular components that act as antigens. Administration of the vaccine stimulates the production of antibodies with specific protective properties. Administer tetanus toxoid vaccine for wound prophylaxis if the vaccine history is unknown or if fewer than 3 tetanus toxoid immunizations have been administered.

Diphtheria and tetanus toxoids, and acellular pertussis vaccine (Infanrix, Adacel, Boostrix)

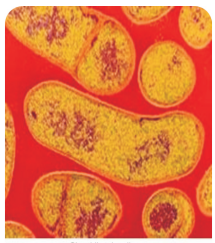
DTaP may be administered into the deltoid or midlateral thigh muscles in children and adults. In infants, the preferred site of administration is the midlateral thigh muscles. This vaccine promotes active immunity to diphtheria, tetanus, and pertussis by inducing the production of specific neutralizing antibodies and antitoxins. It is indicated for active booster immunization for tetanus, diphtheria, and pertussis prevention for persons aged 10-64 years. It is the preferred vaccine for adolescents scheduled for booster.

INTERPRETATION

1

What Are Anaerobic Microorganisms

- Anaerobic microorganisms are widespread and very important
- Do not require oxygen for growth - often extremely toxic


Clostridium botulinum

2

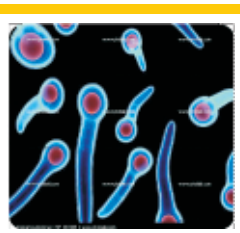
Defining Anaerobes

- Facultative anaerobes - can grow in the presence or absence of oxygen
- Obtain energy by both respiration and fermentation
- Oxygen not toxic, some use nitrate (NO3-) or sulphate (SO42-) as a terminal electron acceptor under anaerobic conditions

3

Strict Anaerobic Bacteria

- Obligate (strict) anaerobes - oxygen is toxic to these organisms, do not use oxygen as terminal electron acceptor.
- Archaea such as methanogens and Bacteria, e.g Clostridia, Bacteriodes etc. etc.



4

Culturing of anaerobes need special skills

- Culture of anaerobes is extremely difficult because need to exclude oxygen, slow growth and complex growth requirements
- By molecular methods based on DNA analysis and direct microscopy have shown that anaerobic bacteria are diverse

5

The accepted specimens for anaerobic processing are as follows:

Sites	Acceptable specimen
CNS	CSF, abscess, tissue
Dental/ENT	Abscess, aspirates, tissues

6

The accepted specimens for anaerobic processing are as follows:

- Local abscess
  - Needle aspirates
- Pulmonary
  - Trans tracheal aspirates, lung aspirates, pleural fluid, tissu
  - Protected bronchial washing

7

The accepted specimens for anaerobic processing are as follows:

- Abdominal
- Urinary tract
- Genital tract
- Ulcers/wounds
- Others

- Abdominal Abscess aspirate, fluid and tissues
- Suprapubic bladder aspirate
- Culdocentesis specimen, endometrial swabs
- Aspirate/swab pus from deep pockets or from under skin flaps that have been decontaminated
- Deep tissue or bone lesions, blood, bone marrow, synovial fluid.
- Tissues

8

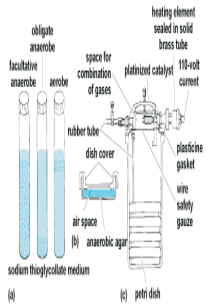
Interpretation by Physicians and Microbiologists

- The physician who collected the specimen can best evaluate the anaerobic culture result.
- Interpretation of the result should be correlated with the clinical findings and how the specimen was collected.
- Clinical signs suggesting possible infection with anaerobes include the following:
  1. Foul smelling discharge
  2. Infection in proximity to a mucosal surface
  3. Gas in tissues
  4. Negative aerobic cultures of specimens pus cells.

9

Testing for anaerobes in Routine Practice

- Deep culture tubes can be used to test whether an unknown organism is anaerobic/facultative or aerobic
- Thiglyclolate added to culture medium, oxygen only found near top where it can diffuse from air -pattern of colony formation characteristic of organisms based on DNA analysis and direct microscopy have shown that anaerobic bacteria are diverse



10

Why Needle Aspiration Preferred for Anaerobic Bacteria

III. Collection by needle aspiration is preferable than swab culture because of

a) better survival of pathogen
b) greater quantity of specimen
c) less contamination with extraneous organism are often achieved

11

B. HANDLING

If a swab must be used, a 2 tube system must be used

→ 1st tube contains swab in O_2 free CO_2

→ 2nd tube contains PRAS (pre-reduced anaerobically sterilized culture media)

Specimen should be placed in anaerobic transport device with gas mixture

12

HANDING AND TRANSPORT OF CLINICAL SPECIMENS

- The basic principles to remember are
  - avoid contamination with the normal microbial flora
  - prompt transport to the laboratory
  - immediate processing is done.

13

Transporting Bacteria

- Anaerobic transport tubes and/or devices should always be available at the OR and ER.
- Specimens should be placed
  - ★ in leak-proof container with tight fitting caps.
  - ★ proper label for identification with date and time of collection should accompany all specimens submitted for culture.
  - ★ Put samples in room temperature while waiting for delivery to the laboratory. Some anaerobes are killed by refrigeration.

14

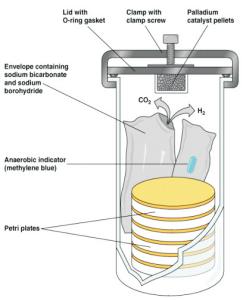
Anaerobic culturing Needs Define Chemicals and Environment

- Pyrogallic acid-sodium hydroxide method can be used, again relies on a chemical reaction to generate an anaerobic environment, but a catalyst rather than a reducing agent
- Anaerobic jars (GasPak System) are sued to incubate plates in an anaerobic atmosphere, useful if brief exposure to oxygen is not lethal

15

Anaerobic Culture Methods

- **Production of a vacuum**
- **Displacement of Oxygen with other gases**
- **Absorption of Oxygen by chemical or biological methods**
- **By using reducing agents**



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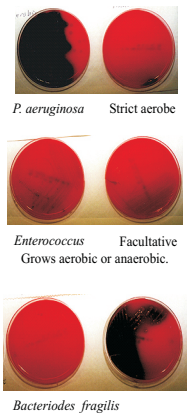
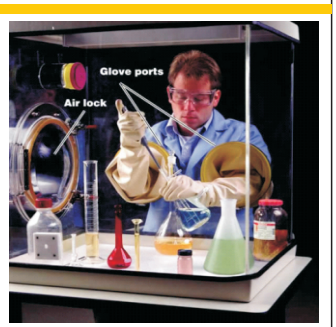


17

Obligate Anaerobes needs Optimal Methods

- **Obligate anaerobes can be culture in special reducing media such as sodium Thiglyclolate or in anaerobe chambers and handled in anaerobe hoods.**



18

Displacement of Oxygen

By inert gases like Hydrogen, Nitrogen, Carbon dioxide or Helium

Use of lighted candle - Use up Oxygen, but some Oxygen is left behind Vacuum decicator - Unsatisfactory

19

McIntosh & Filde's Jar

- Hydrogen gas is passed in
- Catalyst helps to combine Hydrogen & O2
- Reduced Methylene blue remains colorless if anaerobiosis is achieved

20

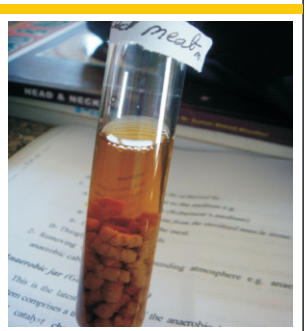
Absorption of O_2 by Chemical method

- Pyrogallol
- Chromium and sulphuric acid
- Gas-pak available commercially

21

By reducing agents

- Thiglyclolate broth
- Robertson's Cooked Meat (RCM) broth contains nutrient broth with pieces of fat-free minced cooked meat of ox heart.



22

McIntosh & Filde's anaerobic Jar

- Stout glass or metal jar with a lid
- Lid has an inlet for gas, outlet & 2 terminals
- Alumina pellets coated with palladium (catalyst) under the lid
- Inoculated plates kept inside the jar
- Lid is clamped tight
- Air is evacuated

23

A solid or liquid medium maybe used & must provide an anaerobic environment Anaerobic Culture System

ANAEROBIC JAR

1. Candle Jar
   - reduces O_2 environment
   - only ↑ CO_2 tension
2. Gas Pak Jar
   a. Palladium aluminum coated pellets
      - catalyst
      - chemically reduces O_2
      - reacts with residual O_2 in the presence of H_2 to form H_2O

24

Culture of strict anaerobes

- For culture of strict anaerobes all traces of oxygen must be removed from medium and for many organisms sample must be kept entirely anaerobic during manipulations
- Methanogenic archaea from rumen and sewage treatment plants killed by even a brief exposure to O_2
- Medium usually boiled during preparation and reducing agent added, stored under O_2-free atmosphere

25

Choosing the Optimal Media

Broth and solid media should both be inoculated. The culture media should include anaerobic blood agar plates enriched with substances such as brain-heart infusion, yeast extract, amino acids, and vitamin K; a selective medium such as kanamycin-vancomycin (KV) blood agar or laked blood agar; and a broth such as brain heart infusion broth with Thiglyclolate or other reducing agent.

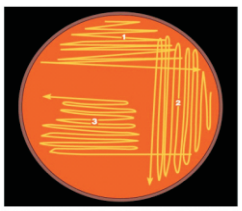
26

Media chosen according to our needs

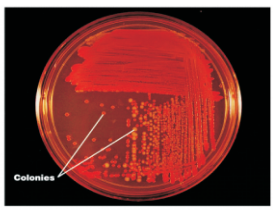
- The choice of media depends upon the type of specimen. Some commonly used media include prereduced peptone-yeast extract-glucose broth which is suitable for analysis of volatile products by gas chromatography; egg yolk agar for detection of lecithinase activity of Clostridium spp.; cycloserine-cefoxitin-fructose agar (CCFA) for isolation of Clostridium difficile from stool; and Bacteroides bile esculin agar for isolation of the Bacteroides fragilis group.

27

A skilled plating the Medium is highly essential



(a) The direction of streaking is indicated by arrows. Streak series 1 is made from the original bacterial culture. The inoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns.



(b) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and yellow, have been obtained.

28

Anaerobic Glove Chamber

b. Gas Pak envelope
   - generates CO_2 & H_2 gases
c. Methylene blue strip
   - indicator
   blue → (O_2)
   white → (−) O_2

II. Anaerobic Glove Chamber
- close system
- used for premature babies
- e.g. incubator

III. Roll Tube
- has a pedal gas (CO_2 & H_2) would come out
- place test tube directly to the outlet

29

IDENTIFICATION of ANAEROBES

Plates are checked at
- > 18-24 hours for faster growing species like Cl. Perfringens & B.fragilis & daily thereafter up to
- > 5-7 days for slowly growing species like Actinomyces, Eubacterium & Propionibacterium

Genus is determined by
- gram stain, cellular morphology, Gas-liquid chromatography

Species determination is based on fermentation of sugars & other biochemical determination

30

Identification of Anaerobes is Complex

- The identification of anaerobes is highly complex, and laboratories may use different identification systems. Partial identification is often the goal. For example, there are six species of the Bactericides genus that may be identified as the Bactericides fragilis group rather than identified individually. Organisms are identified by their colonial and microscopic morphology, growth on selective media, oxygen tolerance, and biochemical characteristics.

31

All isolates to the Purified by Sub culturing

- Isolated organisms are always subcultured and the pure culture is tested in order to identify the organism. The identification of anaerobes is highly complex, and laboratories may use different identification systems. Partial identification is often the goal. For example, there are six species of the Bacteroides genus that may be identified as the Bacteroides fragilis group rather than identified individually. Organisms are identified by their colonial and microscopic examination.

32

Needs several Biochemical Tests for Identification

Organisms are identified by their colonial and microscopic morphology, growth on selective media, oxygen tolerance, and biochemical characteristics. These include sugar fermentation, bile solubility, esculin, starch, and gelatin hydrolysis, casein and gelatin digestion, catalase, lipase, lecithinase, and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography, and susceptibility to antibiotics. The antibiotic susceptibility profile is determined by the micro tube broth dilution method. Many species of anaerobes are resistant to penicillin, and some are resistant to clindamycin and other commonly used antibiotics

33

Antibiotic Sensitivity Testing

.The antibiotic susceptibility profile is determined by the micro tube broth dilution method. Many species of anaerobes are resistant to penicillin, and some are resistant to clindamycin and other commonly used antibiotics

BOUQUET

Brain Teasers

1. **What are the drawbacks of radioimmunoassay systems?**
   A. Low sensitivity
   B. Disposal issues, health hazard pertaining to radioactivity
   C. Older technology
   D. All of the above.

2. **What are the limitations of enzyme immunoassay systems?**
   A. Limitation of photometric measuring range
   B. Low sensitivity in 2nd generation assays
   C. Smaller dynamic range and linearity
   D. All of the above.

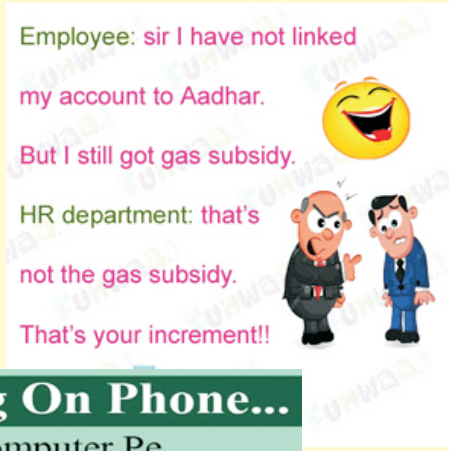
3. **What are the limitations of fluorescence immunoassay systems?**
   A. Compromised sensitivity
   B. Background fluorescence
   C. Protein quenching
   D. All of the above.

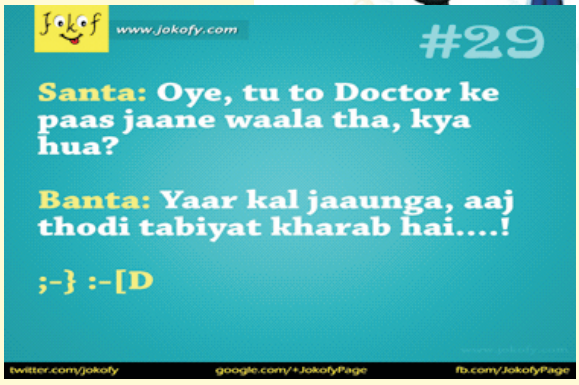
4. **What are the advantages of chemiluminescence technology?**
   A. Good linearity
   B. Good stability
   C. Good sensitivity
   D. All of the above.

ANSWER: 1. D, 2. D, 3. D, 4. D

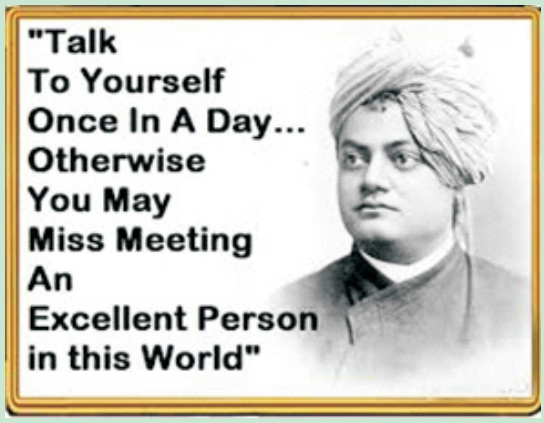
BOUQUET

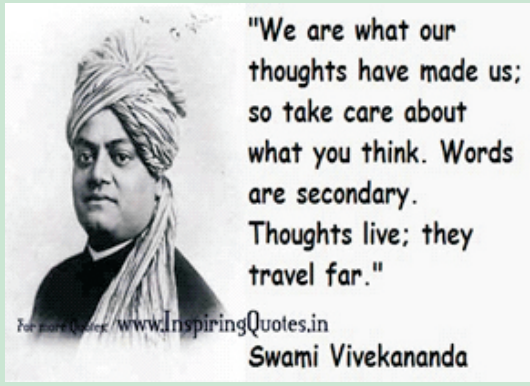
In Lighter Vein

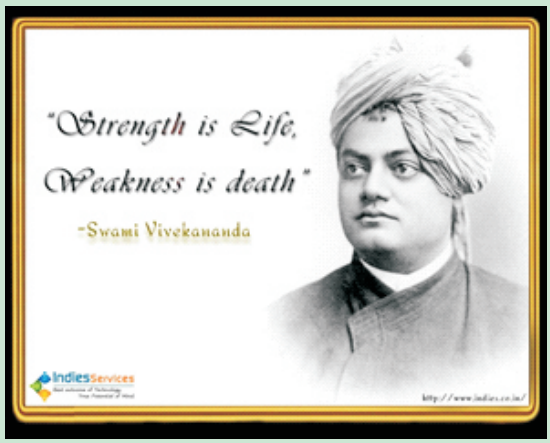


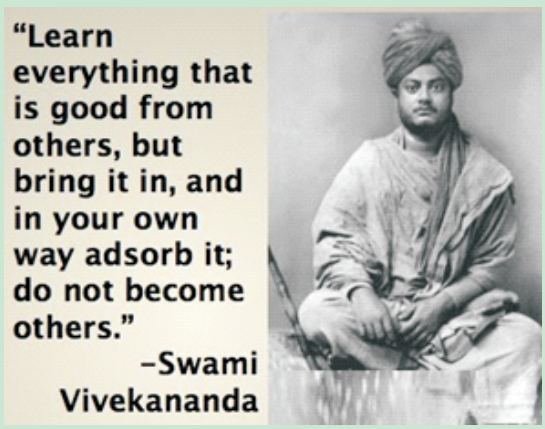


Wisdom Whispers









TROUBLESHOOTING

Anaerobic bacteria culture

Definition

An anaerobic bacteria culture is a method used to grow anaerobes from a clinical specimen. Obligate anaerobes are bacteria that can live only in the absence of oxygen. Obligate anaerobes are destroyed when exposed to the atmosphere for as briefly as 10 minutes. Some anaerobes are tolerant to small amounts of oxygen. Facultative anaerobes are those organisms that will grow with or without oxygen. The methods of obtaining specimens for anaerobic culture and the culturing procedure are performed to ensure that the organisms are protected from oxygen.

Purpose

Anaerobic bacterial cultures are performed to identify bacteria that grow only in the absence of oxygen and which may cause human infection. If overlooked or killed by exposure to oxygen, anaerobic infections result in such serious consequences as **amputation** , organ failure, sepsis, meningitis, and death. Culture is required to correctly identify anaerobic pathogens and institute effective antibiotic treatment.

Precautions

It is crucial that the health care provider obtain the sample for culture via **aseptic technique** . Anaerobes are commonly found on mucous membranes and other sites such as the vagina and oral cavity. Therefore, specimens likely to be contaminated with these organisms should not be submitted for culture (e.g., a throat or vaginal swab). Some types of specimens should always be cultured for anaerobes if an infection is suspected. These include abscesses, bites, blood, cerebrospinal fluid and exudative body fluids, deep wounds, and dead tissues. The specimen must be protected from oxygen during collection and transport and must be transported to the laboratory immediately.

Description

Anaerobes are normally found within certain areas of the body but result in serious infection when they have access to a normally sterile body fluid or deep tissue that is poorly oxygenated. Some anaerobes normally live in the crevices of the skin, in the nose, mouth, throat, intestine, and vagina. Injury to these tissues (i.e., cuts, puncture wounds, or trauma) especially at or adjacent to the mucous membranes allows anaerobes entry into otherwise sterile areas of the body and is the primary cause of anaerobic infection. A second source of anaerobic infection occurs from the introduction of spores into a normally sterile site. Spore-producing anaerobes live in the soil and water, and spores may be introduced via wounds, especially punctures. Anaerobic infections are most likely to be found in persons who are immunosuppressed, those treated recently with broad-spectrum **antibiotics** , and persons who have a decaying tissue injury on or near a mucous membrane, especially if the site is foul-smelling.

Some specimens from which anaerobes are likely to be isolated are:

- blood
- bile
- bone marrow
- cerebrospinal fluid
- direct lung aspirate
- tissue biopsy from a normally sterile site
- fluid from a normally sterile site (like a joint)
- dental abscess
- abdominal or pelvic abscess
- knife, gunshot, or surgical wound
- severe burn

Some of the specimens that are not suitable for anaerobic cultures include:

- coughed throat discharge (sputum)
- rectal swab
- nasal or throat swab
- urethral swab
- voided urine

Specimen collection

The keys to effective anaerobic bacteria cultures include collecting a contamination-free specimen and protecting it from oxygen exposure. Anaerobic bacteria cultures should be obtained from an appropriate site without the health care professional contaminating the sample with bacteria from the adjacent skin, mucus membrane, or tissue. Swabs should be avoided when collecting specimens for anaerobic culture because cotton fibers may be detrimental to anaerobes. Abscesses or fluids can be aspirated using a sterile syringe that is then tightly capped to prevent entry of air. Tissue samples should be placed into a degassed bag and sealed, or into a gassed out screw top vial that may contain oxygen-free prereduced culture medium and tightly capped. The specimens should be plated as rapidly as possible onto culture media that has been prepared.

Culture

Cultures should be placed in an environment that is free of oxygen, at 95°F (35°C) for at least 48 hours before the plates are examined for growth. Gram staining is performed on the specimen at the time of culture. While infections can be caused by aerobic or anaerobic bacteria or a mixture of both, some infections have a high probability of being caused by anaerobic bacteria. These infections include brain abscesses, lung abscesses, aspiration pneumonia, and dental infections. Anaerobic organisms can often be suspected because many anaerobes have characteristic microscopic morphology (appearance). For example, *Bacteroides* spp. are gram-negative rods that are pleomorphic (variable in size and shape) and exhibit irregular bipolar staining. *Fusobacterium* spp. are often pale gram-negative spindle-shaped rods having pointed ends. *Clostridium* spp. are large gram-positive rods that form spores. The location of the spore (central, subterminal, terminal, or absent) is a useful differential characteristic. The presence of growth, oxygen tolerance, and Gram stain results are sufficient to establish a diagnosis of an anaerobic infection and begin antibiotic treatment with a drug appropriate for most anaerobes such as clindamycin, metronidazole, or vancomycin.

Gram-negative anaerobes and some of the infections they produce include the following genera:

- *Bacteroides* (the most commonly found anaerobes in cultures; intra-abdominal infections, rectal abscesses, soft tissue infections, liver infection)
- *Fusobacterium* (abscesses, wound infections, pulmonary and intracranial infections)
- *Porphyromonas* (aspiration pneumonia, periodontitis)
- *Prevotella* (intra-abdominal infections, soft tissue infections)

Gram-positive anaerobes include the following:

- *Actinomyces* (head, neck, pelvic infections; aspiration pneumonia)
- *Bifidobacterium* (ear infections, abdominal infections)
- *Clostridium* (gas, gangrene, food poisoning, tetanus, pseudomembranous colitis)
- *Peptostreptococcus* (oral, respiratory, and intra-abdominal infections)
- *Propionibacterium* (shunt infections)

The identification of anaerobes is highly complex, and laboratories may use different identification systems. Partial identification is often the goal. For example, there are six species of the *Bacteroides* genus that may be identified as the *Bacteroides fragilis* group rather than identified individually. Organisms are identified by their colonial and microscopic morphology, growth on selective media, oxygen tolerance, and biochemical characteristics. These include sugar fermentation, bile solubility, esculin, starch, and gelatin hydrolysis, casein and gelatin digestion, catalase, lipase, lecithinase, and indole production, nitrate reduction, volatile fatty acids as determined by gas chromatography, and susceptibility to antibiotics. The antibiotic susceptibility profile is determined by the microtube broth dilution method. Many species of anaerobes are resistant to penicillin, and some are resistant to clindamycin and other commonly used antibiotics.

Diagnosis/Preparation

The health care provider should take special care to collect a contamination-free specimen. All procedures must be performed aseptically. The health care professional who collects the specimen should be prepared to take two samples, one for anaerobic culture and one for aerobic culture, since it is unknown whether the pathogen can grow with or without oxygen. In addition, health care professionals should document any antibiotics that the patient is currently taking and any medical conditions that could influence growth of bacteria.

Aftercare

In the case of vein puncture for anaerobic blood cultures, direct pressure should be applied to the vein puncture site for several minutes or until the bleeding has stopped. An adhesive bandage may be applied, if appropriate. If swelling or bruising occurs, ice can be applied to the site. For collection of specimens other than blood, the patient and the collection site should be monitored for any complications after the procedure.

Risks

Special care must be taken by the health care team obtaining, transporting, and preparing the specimen for anaerobic culture. Poor methodology may delay the identification of the bacterium, may allow the patient's condition to deteriorate, and may require the patient to provide more samples than would otherwise be required. Patients may experience bruising, discomfort, or swelling at the collection site when tissue, blood, or other fluids are obtained.

Results

Negative results will show no pathogenic growth in the sample. Positive results will show growth, the identification of each specific bacterium, and its antibiotic susceptibility profile.

Patient education

A health care team member should explain the specimen collection procedure to the patient. If the patient is seriously ill, the team member should explain the procedure to the patient's family members. The patient and his or her family should understand that because bacteria need time to grow in the laboratory, several days may be required for bacterium identification.

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