MENINGITIS
ENCEPHALITIS

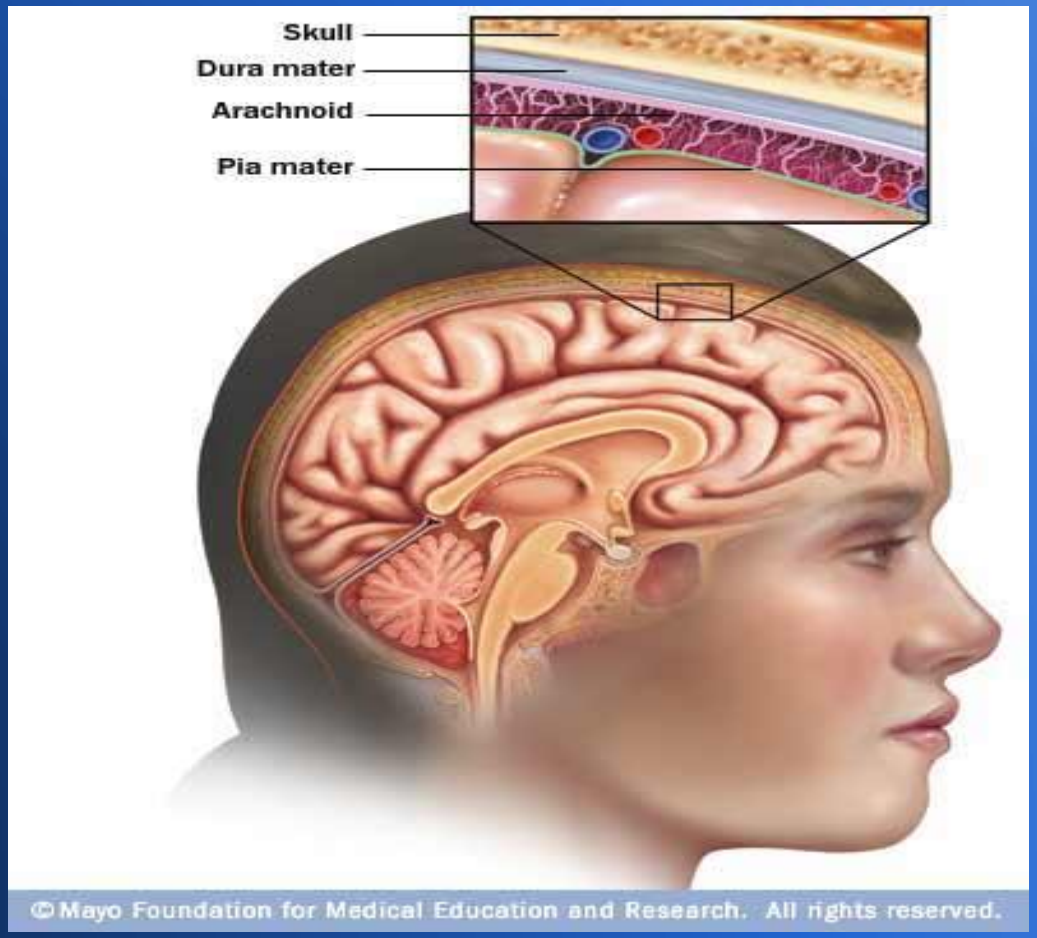
Meningitis

Meningitis is inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges

Clinically, this characteristically results in the occurrence of :

-meningeal symptoms -headache, nuchal rigidity, photophobia

- increased number of white blood cells in the cerebrospinal fluid - CSF; pleocytosis.



Etiology

Infectious causes:

- bacterial meningitis – *Streptococcus pneumoniae* , Neisseria meningitidis, *Haemophilus influenzae,* Mycobacterium tuberculosis
- viral meningitis - Enteroviruses, Mumps virus, Herpes virus, Influenza A and B viruses, Rubella virus, HIV
- fungal -Cryptococcus neoformans, *Histoplasma* capsulatum, Candida spp
- spirochetes -Treponema pallidum,Leptospira spp.,Borrelia burgdorferi,Borrelia recurrentis
- rickettsiae
- parasitic meningitis - Toxoplasma gondii, Trichinella spiralis,Tænia solium,Entamoeba histolytica

Noninfectious causes:

- medications - nonsteroidal anti-inflammatory drugs, antibiotics-ciprofloxacin, TMP-SMX
- systemic illness- Systemic lupus erythematosus, Sarcoidosis
- carcinomatosis

Epidemiology

BACTERIA

N meningitidis - --> children and young adults. Patients with imune deficiencies --->recurrent disseminated infections.

H. Influenzae Type B ---> infants and children younger than 6 years

S. pneumoniae ---> adults with pneumococcal infection (otitis media, mastoiditis sinusitis, pneumonia, endocarditis) or in patients who have suffered a basilar skull fracture with CSF leak.

Listeria monocytogenes ---> neonates (pregnant women may harbor the organism asymptomatically in their genital tract).

Tuberculosis is associated with active, progressive systemic disease -most common cause of chronic meningitis

VIRUS (aseptic meningitis)

Enteroviruses --->spread by the fecal-oral route

Mumps virus in an unimmunized population most common causes of aseptic meningitis and encephalitis, in the winter and spring months. The peak incidence is in children ages 5 – 9 years.

Lymphocytic choriomeningitis transmitted to humans by contact with rodents or their excreta

Herpes viruses

HIV - persist in the CNS after initial infection.

FUNGI

Cryptococcus neoformans--->most common patients with AIDS

EPIDEMIOLOGY

- sporadic diseases or small epidemics

- Source of infection:

man (carrier, ill man)

animals (viral, leptospirotic meningitis).

- Transmission:

Airborne (meningococcus),

Fecal oral route (enteroviruses),

Cutaneo-mucous (leptospirotic, herpes simplex meningitis).

- Receptivity: depends on age, imune system.

- Immunity weak or absent

Pathophysiology

Three major pathways exists by which an infectious agent gains access to the central nervous system (CNS) and causes disease.

- initially, colonization or a localized infection - skin, nasopharynx, respiratory tract, gastrointestinal tract, or genitourinary tract. Most meningeal pathogens are transmitted through the respiratory route, as exemplified by the nasopharyngeal carriage of *Neisseria meningitides* (meningococcus) and nasopharyngeal colonization with *S pneumoniae* (pneumococcus).

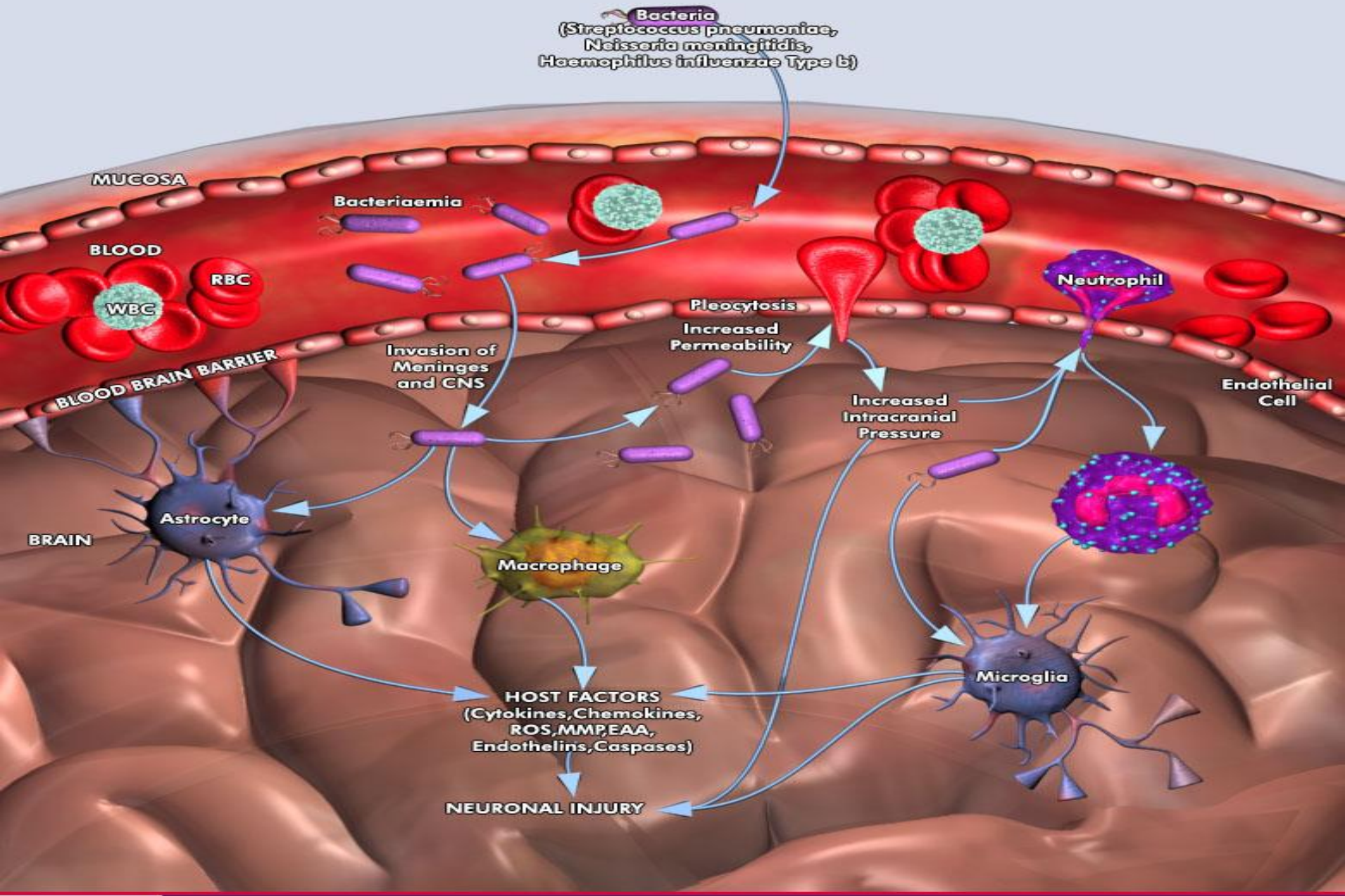
- from this site, invadation the submucosa, access to the CNS by

(1) invasion of the bloodstream (ie, bacteremia, viremia, fungemia, parasitemia) and subsequent hematogenous seeding of the CNS;

(2) a retrograde neuronal (ie, olfactory and peripheral nerves) pathway;

(3) direct contiguous spread (ie, sinusitis, otitis media, congenital malformations, trauma, direct inoculation during intracranial manipulation).

Bacterial Meningitis



Pathophysiology

-once inside the CNS, the infectious agents likely survive because host defenses (eg, immunoglobulins, neutrophils, complement components) appear to be limited here , the infectious agents incite a cascade of meningeal inflammation (TNF-alpha and IL-1 are the most prominent among the cytokines that mediate this inflammatory cascade)

- the result is vascular endothelial injury and increased blood-brain-barrier permeability ----> elevated CSF protein levels, neutrophilic pleocytosis

- the ensuing cerebral edema (ie, vasogenic, cytotoxic, interstitial) significantly contributes to intracranial hypertension and a consequent decrease in cerebral blood flow- anaerobic metabolism -increased lactate concentration and hypoglycorrhachia

The Most Common Bacterial Pathogens Based on Age and Predisposing Risks

Age 0-4 weeks -S agalactiae (group B streptococci),E coli K1,L monocytogenes

Age 4-12 weeks- S agalactiae, E coli H influenzae S pneumoniae N meningitidis

Age 3 months to 18 years - N meningitidis, S pneumoniae, H influenzae

Age 18-50 years - S pneumoniae, N meningitidis, H influenzae

Age older than 50 years -S pneumoniae, N meningitidis, L monocytogenes Aerobic gram-negative bacilli

Immunocompromised state - S pneumoniae, N meningitidis, L monocytogenes Aerobic gram-negative bacilli

Intracranial manipulation, including neurosurgery - Staphylococcus aureus Coagulase-negative staphylococci, Aerobic gram-negative bacilli, including Pseudomonas aeruginosa

Basilar skull fracture - S pneumoniae, H influenzae Group A streptococci

CSF shunts - Coagulase-negative staphylococci S aureus Aerobic gram-negative bacilli, Propionibacterium acnes

The classic presentation of meningitis

- includes fever, headache, signs of meningeal irritation, photophobia, nausea, vomiting, and signs of cerebral dysfunction (eg, lethargy, confusion, coma).
- atypical presentation- absence of meningeal signs-
  - in elderly individuals with underlying comorbidities (eg, diabetes, renal and liver disease)
  - patients with neutropenia, including organ and tissue transplant recipients
  - patients with HIV and AIDS

Clinical

A. Infectious syndrome: fever, chills, myalgia;

B. Intracranial hypertension syndrome

Headache, vomiting, lethargy, bradicardia,seizures, changes in alert level

Papilledema- eye examination - risk of brain herniation after spinal tap

***C.* Encephalitic syndrome**

Signs of cerebral dysfunction- confusion, irritability, delirium, and coma- accompanied by fever and photophobia.

Altered mental status → coma, generalized seizures, hallucinations and psychomotor agitation,piramidal ,extrapiramidal, brainstem and cerebelous syndrome, reflex impairement

Physical

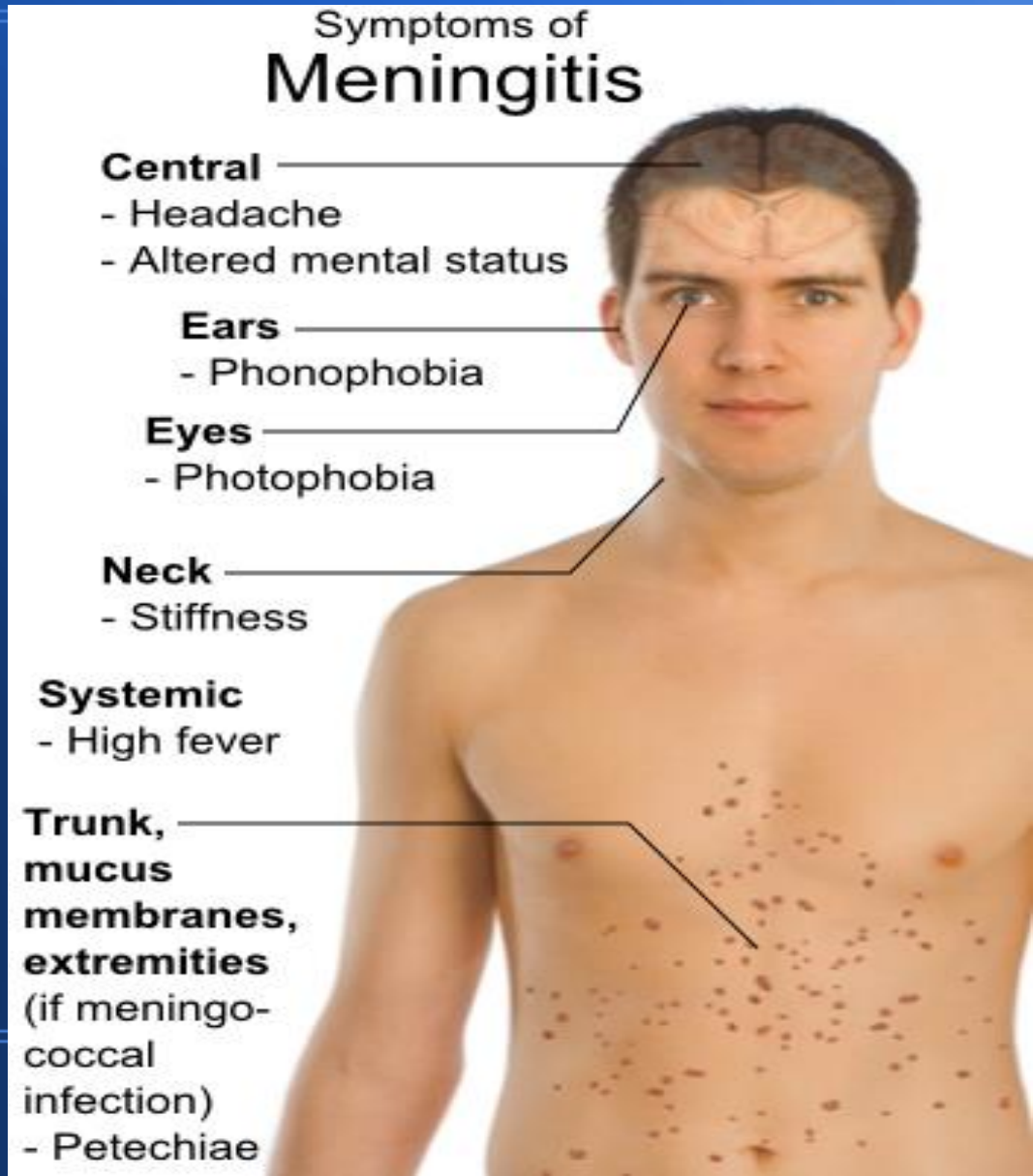
D. Meningean syndrome:

- Simptoms:

- headache (violent, continuous or with paroxisms)
- radiculopathy (lumbar radiating pain to the lower extremities)
- vomiting
- photophobia

The pacient adopts "antalgic positions", for lowering the pain

- Lateral decubitus- triple flexion
- "tripod position", sitting

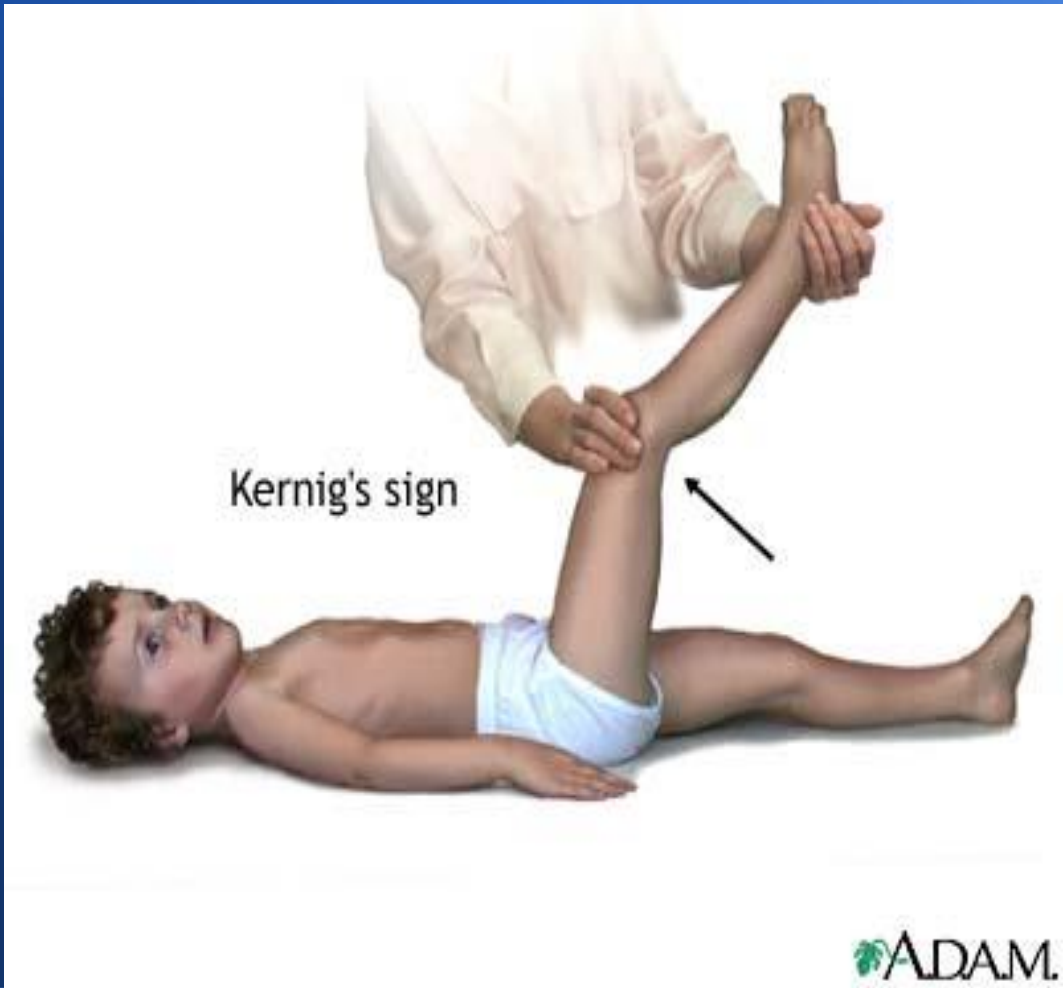


MENINGEAN SYNDROME
Signs

Kernig sign: In a supine patient, flex the hip to 90° while the knee is flexed at 90°. An attempt to further extend the knee produces pain in the hamstrings and resistance to further extension.

Nuchal Rigidity

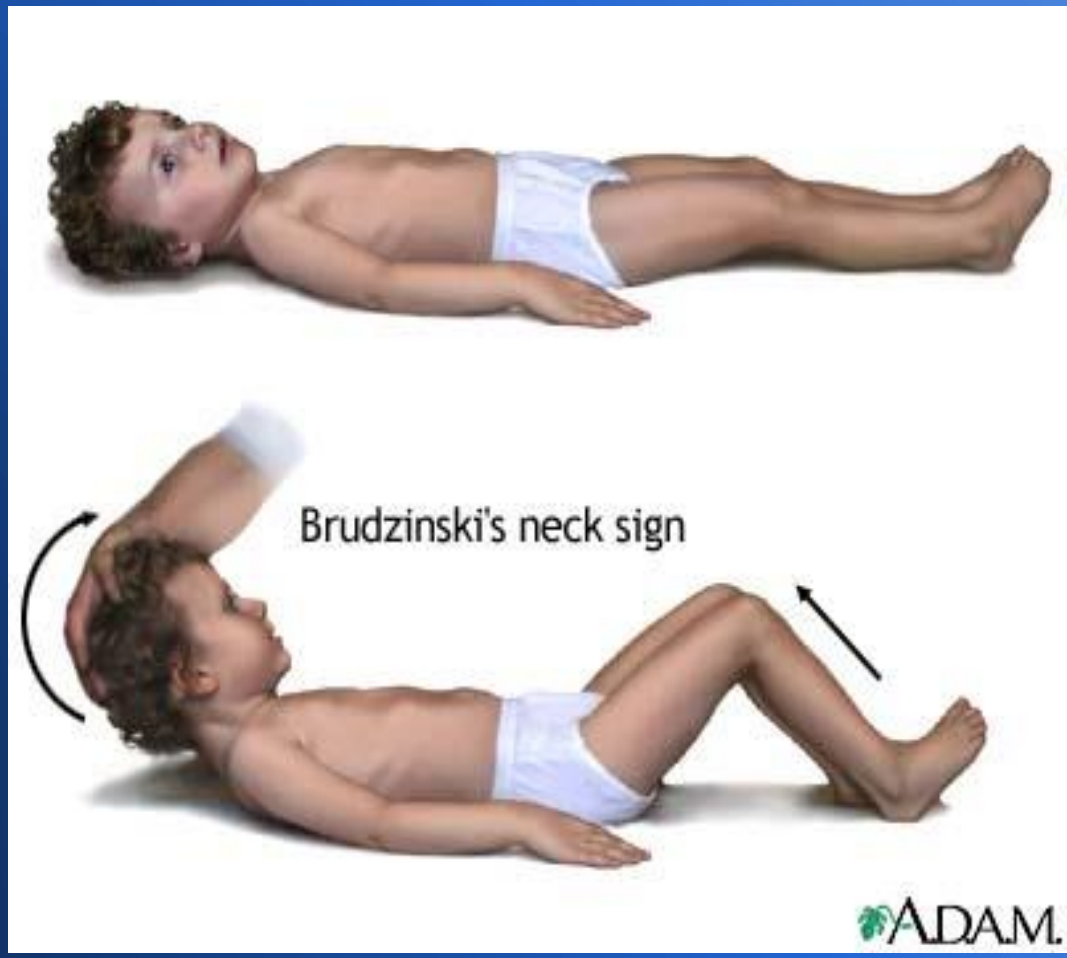
1. Involuntary muscle spasm limits passive neck flexion
2. Patient cannot flex neck to place chin on chest



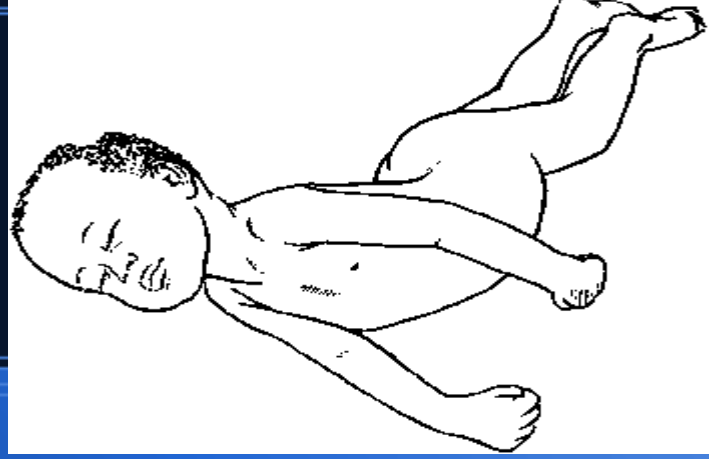
MENINGEAN SYNDROME
Signs

Brudzinski sign: Passively flex the neck while the patient is in a supine position with extremities extended. This maneuver produces flexion of the hips in patients with meningeal irritation.

Nuchal rigidity: Resistance to passive flexion of the neck is also a sign.



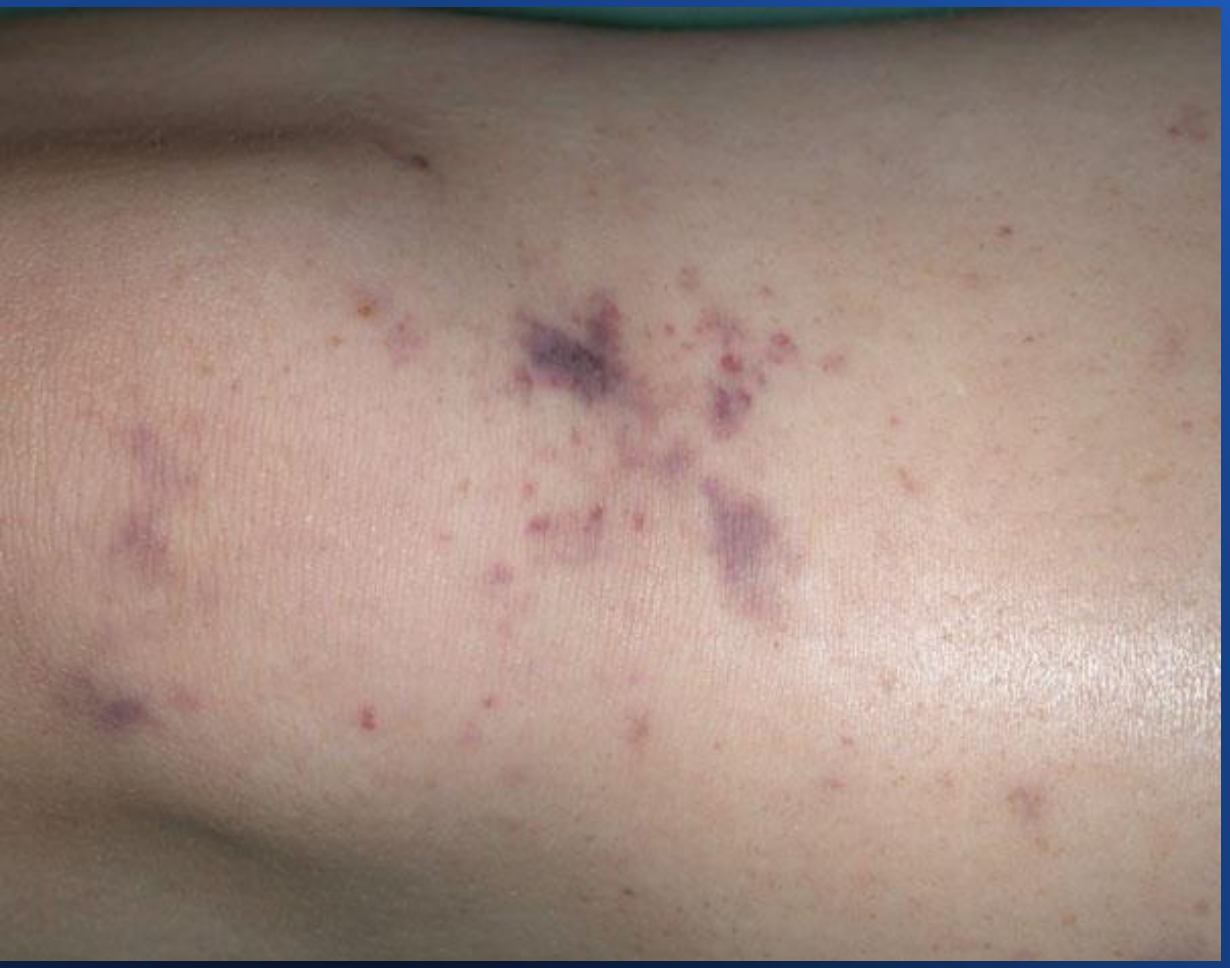
Newborn Meningitis



In the newborn, signs and symptoms of bacterial meningitis are often very similar to those of sepsis or other serious illnesses.

- Neonates with acute bacterial meningitis often lack meningismus. Meningitis may manifest as hyperthermia or hypothermia, poor feeding, lethargy, irritability, vomiting, or respiratory distress. A bulging fontanelle may be seen in up to one-third of cases, although it usually appears later in the course of illness.
- Causes
  - Bacterial meningitis: group B Streptococcus 50%,E coli 20%,Listeria monocytogenes 5-10%
  - HSV and VZV meningitis
- Opisthotonus in the neonate may be a symptom of meningitis, tetanus, or severe kernicterus.

Rash in meningococcal meningitis



A fever + spots or a rash that do not fade under pressure= a medical emergency – meningococcemia- meningitis + hemorrhagic rashes/ spots

Meningeal irritation
Meningismus

Causes of meningeal irritation

1. Meningitis
2. Meningismus
3. Subarachnoid Hemorrhage
4. Posterior fossa tumor
5. Increased Intracranial Pressure

Meningismus may develop in young children with pneumonia, Shigella infections, rubella, influenza. There is elevated intracranial pressure (headache, vomiting, stiff neck) but in CSF pleyocitosis or biochemical changes are not present.

Lumbar puncture should be performed promptly if there is clinical manifestation suggesting meningeal irritation.

CLUES TO THE ETIOLOGY

Morbilliform rash with pharyngitis and adenopathy - a viral etiology (eg, Epstein-Barr virus, cytomegalovirus, adenovirus, HIV).

Macules and petechiae that rapidly evolve into purpura located principally on the extremities, suggest meningococcemia

Vesicular lesions in a dermatomal distribution suggest varicella-zoster virus. Genital vesicles suggest HSV-2 meningitis.

Sinusitis or otitis suggests direct extension into the meninges- *S pneumoniae* and *H influenzae.*

Rhinorrhea or otorrhea suggests a CSF leak from a basilar skull fracture, with meningitis most commonly caused by S pneumoniae.

The presence of a murmur suggests infective endocarditis with secondary bacterial seeding of the meninges.

Evidence of parotitis is observed in some cases of mumps meningitis

Aseptic meningitis

Bacteria	Partially-treated bacterial meningitis L monocytogenes Brucella species Rickettsia rickettsii Ehrlichia species Mycoplasma pneumoniae B burgdorferi Treponema pallidum Leptospira species Mycobacterium tuberculosis Nocardia species
Parasites	N fowleri Acanthamoeba species Balamuthia species Angiostrongylus cantonensis G spinigerum Baylisascaris procyonis S stercoralis Taenia solium (cysticercosis)
Fungi	Cryptococcus neoformans C immitis B dermatitidis H capsulatum Candida species Aspergillus species

Fungi	Cryptococcus neoformans C immitis B dermatitidis H capsulatum Candida species Aspergillus species
Viruses	Enterovirus Poliovirus Echovirus Coxsackievirus A Coxsackievirus B Enterovirus 68-71
	Herpesvirus HSV-1 and HSV-2 Varicella-zoster virus EBV, CMV, HHV*-6, HHV-7
	Paramyxovirus Mumps virus, Measles virus Togavirus, Flavivirus, Alphavirus, Reovirus, Arenavirus, Rhabdovirus, Retrovirus

Tuberculous meningitis

- insidious prodrome characterized by malaise, low-grade fever, intermittent headache, and changing personality. A meningitic phase develops within 2-3 weeks, characterized by headache, vomiting, and confusion. Meningismus and signs of meningeal irritation are absent in 25-40% of cases. Up to 30% of patients have focal neurologic signs, most frequently cranial nerve palsies (cranial nerve VI, III, IV, and VIII) - Basilar meningitis with TB

Always consider tuberculous meningitis in the differential diagnoses of patients with aseptic meningitis or chronic meningitis syndromes!!!

Clinical staging of meningeal tuberculosis is based on neurologic status.

- Stage 1 - no change in mental function with no deficits and no hydrocephalus.
- Stage 2 refers to a patient with confusion and evidence of neurologic deficit.
- Stage 3 refers to an individual with stupor and lethargy.

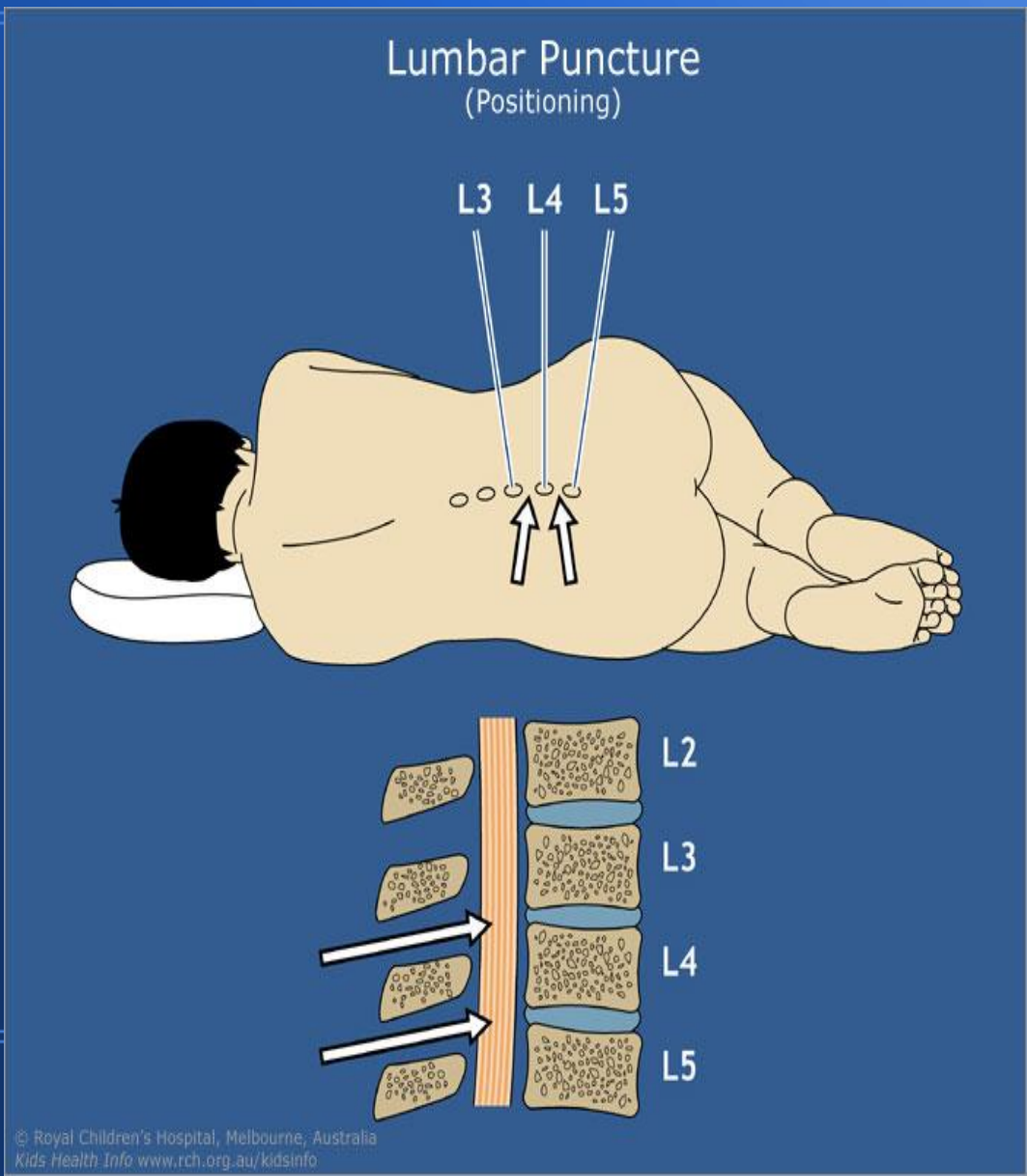
Agent	Opening Pressure	WBC count per µL	Glucose (mg/dL)	Protein (mg/dL)	Microbiology
Bacterial meningitis	200-300 CSF cloudy/ clear	100-5000; >80% PMNs	<40	>100	Specific pathogen demonstrated in 60% of Gram stains and 80% of cultures
Viral meningitis	90-200 CSF clear	10-300; lymphocytes	Normal, reduced in mumps	Normal but may be slightly elevated	Viral isolation, PCR assays
Tuberculous meningitis	180-300 CSF clear/ cloudy	100-500; lymphocytes	Reduced <40	Elevated >100	Acid-fast bacillus stain, culture, PCR
Cryptococcal meningitis	180-300 CSF clear/ cloudy	10-200; lymphocytes	Reduced	50-200	India ink, cryptococcal antigen, culture
Aseptic meningitis	90-200 CSF clear	10-300; lymphocytes	Normal	Normal but may be slightly elevated	Negative findings on workup
Normal values	80-200 CSF clear	0-5; lymphocytes	50-75	15-40	Negative findings on workup

•Contraindications

- INR > 1.4 or other coagulopathy
- trombocytemia < 50.000/mm3
- infection at desired puncture site
- obstructive / non-communicating hydrocephalus

intracranial mass

- high intracranial pressure (ICP) / papilledema
- focal neurological symptoms/signs
- partial / complete spinal block
- acute spinal trauma



CSF examination

- Spinal tap: lombar (L4-L5)
- macroscopic: aspect (CSF clear, hemorrhagic, turbid, purulent, xanthochrom), CSF opening pressure (normotensive, hypo-, hypertensive)
- Pandy reaction: to determine the presence of proteins in the spinal fluid, by adding one drop of spinal fluid to 1 mL of solution (carbolic acid crystals in distilled water, cresol, or pyrogallic acid); the reaction varies from a faint turbidity to a dense “milky” precipitate according to the degree of protein content. (- /++++)
- Cytologia:

Element count/mmc after erytrocyte lisation with acetic acid

CSF examination

- Bacteriologic examination:

a). sediment examination -after CSF centrifugation colored smear (Gram, blue methylen), special (Ziehl Nielsen, China Ink,Giemsa, Field)

b). cultures : blood enriched gelosis (simple, chocolat), Muller-Hinton, Loewenstein, Sabouraud.

- Imunologic tests

a). Contraimunelectroforesis

b). Coaglutination

c). Latex aglutination

d). ELISA

e) PCR

COMPLICATIONS

1) Early complications:

Disease related: Disseminated intravascular coagulation, toxic shock, seizures, cerebral vasculitis, development of venous sinus thrombosis, obstruction of CSF flow, or the formation of subdural empyema and brain abscess., septic metastasis (articular, endocardic, pericardial, pulmonary)

Procedure and treatment related: Bleeding into the spinal canal, brain herniation, alergy, dysmicrobism,catheter infection, seizures.

1) Tardiv complications and sequeles: memory and congnitive impairement,mental retardation, Cranial nerve palsies, motor deficits,learning disabilities, cortical blindness, and deathness, hydrocephaly, epileptic disorders.

Differential diagnosis

- Before spinal tap: febrile intracranian expansive processes (abces, tumors)
- After spinal tap
  - meningismus (meningean syndrome+ normal CSF)
  - non/Infectious disease
- Patogenic differentiation: primar/secundary meningitis
- Etiologic differentiation: viral, bacterian,TB, fungic
- CSF aspect:

1) Clear: viral, TB
2) Turbid/opalescent: bacterian
3) Hemorrhagic : Listeria monocytogenes, Baccilus antracis

Recommended Empiric Antibiotics According to Predisposing Factors for Patients With Suspected Bacterial Meningitis

Predisposing Feature	Antibiotic(s)
Age 0-4 weeks	Ampicillin plus cefotaxime or an aminoglycoside
Age 1-3 months	Ampicillin plus cefotaxime plus vancomycin
Age 3 months to 50 years	Ceftriaxone or cefotaxime plus vancomycin
Older than 50 years	Ampicillin plus ceftriaxone or cefotaxime plus vancomycin
Impaired cellular immunity	Ampicillin plus ceftazidime plus vancomycin
Neurosurgery, head trauma, or CSF shunt	Vancomycin plus ceftazidime

Recommended Empiric Antibiotics for Patients With Suspected Bacterial Meningitis and Known CSF Gram Stain Results

Gram Stain Morphology	Antibiotic(s)
Gram-positive cocci	Vancomycin plus ceftriaxone or cefotaxime
Gram-negative cocci	Penicillin G
Gram-positive bacilli	Ampicillin plus an aminoglycoside
Gram-negative bacilli	Broad-spectrum cephalosporin† plus an aminoglycoside

Treatment
Meningitis

Most viral meningitis are benign and self-limited, they require only supportive care and do not require specific therapy.

The antiviral management of herpes meningitis - Acyclovir (10 mg/kg IV q8h) has been administered for HSV-1 and HSV-2 meningitis.

Instituting highly active antiretroviral therapy (HAART) may be necessary for patients with HIV meningitis that occurs during an acute seroconversion syndrome.

Treatment of cryptococcal meningitis (ie, *C neoformans*)

Induction therapy: amphotericin B (0.7-1 mg/kg/d IV) for at least 2 weeks, with or without flucytosine (100 mg/kg PO) in 4 divided doses

Consolidation therapy: fluconazole (400 mg/d for 8 wk).

Maintenance therapy: Long-term antifungal therapy - fluconazole (200 mg/d) to prevent relapse only in patients with AIDS

TB meningitis

Adjunctive therapy

The use of first-line drugs INH 300 mg qd, RIF 600 mg qd, PZM 15-30 mg/kg qd, ethambutol 15-25 mg/kg qd, streptomycin 7.5 mg/kg q12h) - treatment duration at least 12 months

The use of corticosteroids is indicated for individuals with stage 2 or stage 3 disease (ie, patients with evidence of neurologic deficits or changes in their mental function), the dose is 60-80 mg/day of prednisone po, which may be tapered gradually during a span of 6 weeks

Corticosteroids or other antiinflammatory drugs may avert major neurologic sequelae, including hearing loss in children with H. influenzae meningitis.

Hyperosmolar agents (mannitol, hypertonic glucose) are used to reduction of intracranial pressure

Cardiac parameters need to be monitored.

PREVENTION

HIB (Hemophilus influenzae B) vaccination

Vaccinations against encapsulated bacterial organisms (eg, *S pneumoniae, N meningitidis*) - functional or structural asplenia; always administer to individuals who undergo splenectomy.

Vaccination against measles and mumps

N meningitides

prophylaxis is suggested for contacts of persons with meningococcal meningitis- household contacts, daycare center members, close contacts in military barracks or boarding schools

rifampin (600 mg PO q12h) for 2 days ; alternative agents include ceftriaxone (250 mg IM) as a single dose in adults/ pregnant patients; Ciprofloxacin (500-750 mg) as a single dose

H influenzae type b

- Rifampin (20 mg/kg/d) for 4 days.

PROGNOSIS

Patients with viral meningitis usually have a good prognosis for recovery.

The prognosis is worse for patients at the extremes of age (ie, <2 y, >60 y) and those with significant comorbidities and underlying immunodeficiency

Bad prognosis:

- Low peripheral WBC (leukopenia)
- Development of the Waterhouse-Friderichsen syndrome
- Delayed therapy

ENCEPHALITIS

What causes encephalitis?

- Viruses (most common)
  - More than 100 different viruses can cause acute encephalitis
  - Seasonal and geographic distribution can help narrow differential diagnosis
  - Examples of common viruses:
    - Arboviruses
    - Enteroviruses
    - Mumps, Varicella
    - Herpes simplex virus
    - Influenza
    - Rabies

What causes encephalitis?

- Bacteria
  - Tuberculosis, cat-scratch disease, Brucellosis, typhoid fever
- Spirochetes
  - Leptospirosis, Syphilis, Lyme disease
- Fungi
  - Cryptococcosis, Histoplasmosis
- Other infections
  - Cerebral malaria, Toxoplasmosis, amoebiasis

Etiology and pathology

Encephalitis may be:

- Primary manifestation of a viral infection (arbovirus, enteroviruses, herpes simplex, varicella-zoster an mumps viruses)
- Secondary complication, with an immunologic mechanism (e.g. secondary to measles, chickenpox, rubella, smallpox vaccination); this is typically develop 5 to 10 days after onset of illness and are characterized by perivascular demyelination.

Viral encephalitis: It is a well-recognized complication of many common viral infection, most cases are mild, self-limiting condition, but infections such as rabies and herpes simplex result in extensive tissue destruction and are often fatal

Examples of viral encephalitis

- Herpes simplex encephalitis: caused by HSV type 1& 2.

HSV infection is common in infants and immunocompromised individuals.

- HIV encephalitis:
  - HIV is a neurotropic virus that causes subacute encephalitis.
  - Clinically patient develops dementia
- Poliomyelitis:
  - The virus infects the meninges, producing acute lymphocytic meningitis.
  - It also infects the lower motor neurons producing acute paralysis of the affected muscles.
  - In acute cases death can occur from paralysis of respiratory muscles

- **Rabies**:
  - It is transmitted to humans by the bite of a rabid animal usually a dog.
  - The virus enters the cutaneous nerve radicles at the site of inoculation and passes proximally to CNS.
  - Rabies virus causes severe necrotising encephalitis

maximally affecting the basal ganglia, midbrain, and fourth ventricles.

  - Clinically the patient presents with fever, generalized convulsions precipitated by air, noise and water.

Slow virus infection (Spongiform encephalopathies):

  - Includes Creutzfeldt-Jacob disease (CJD) and Kuru.
  - This disease is caused by subviral transmissible agent known as a Prion. Prion consists of abnormal protein that is infectious and transmissible.
  - Clinically both the diseases are characterized by dementia, followed by ataxia, which is progressive and fatal
  - The changes seen are

slowly progressing degeneration of the brain with

progressive neuronal loss,
demielination and

spongiform change in the cerebral white matter.

Epidemiology

There are two distinct epidemiologic patterns in viral encephalitis.

- respiratory or oral person-to-person transmission as exemplified by measles virus, mumps virus, enteroviruses, and herpesviruses, including varicella-zoster virus.
- typical of the arboviral diseases, requires inoculation of the virus into the human bloodstream through the bite of an infected insect, usually a mosquito (Flaviviridae, Togaviridae, and Bunyaviridae) or tick (tick-borne encephalitis virus). In the case of rabies virus and monkey herpesvirus B, direct inoculation into neural tissue by the bite of an infected animal is the mode of transmission.

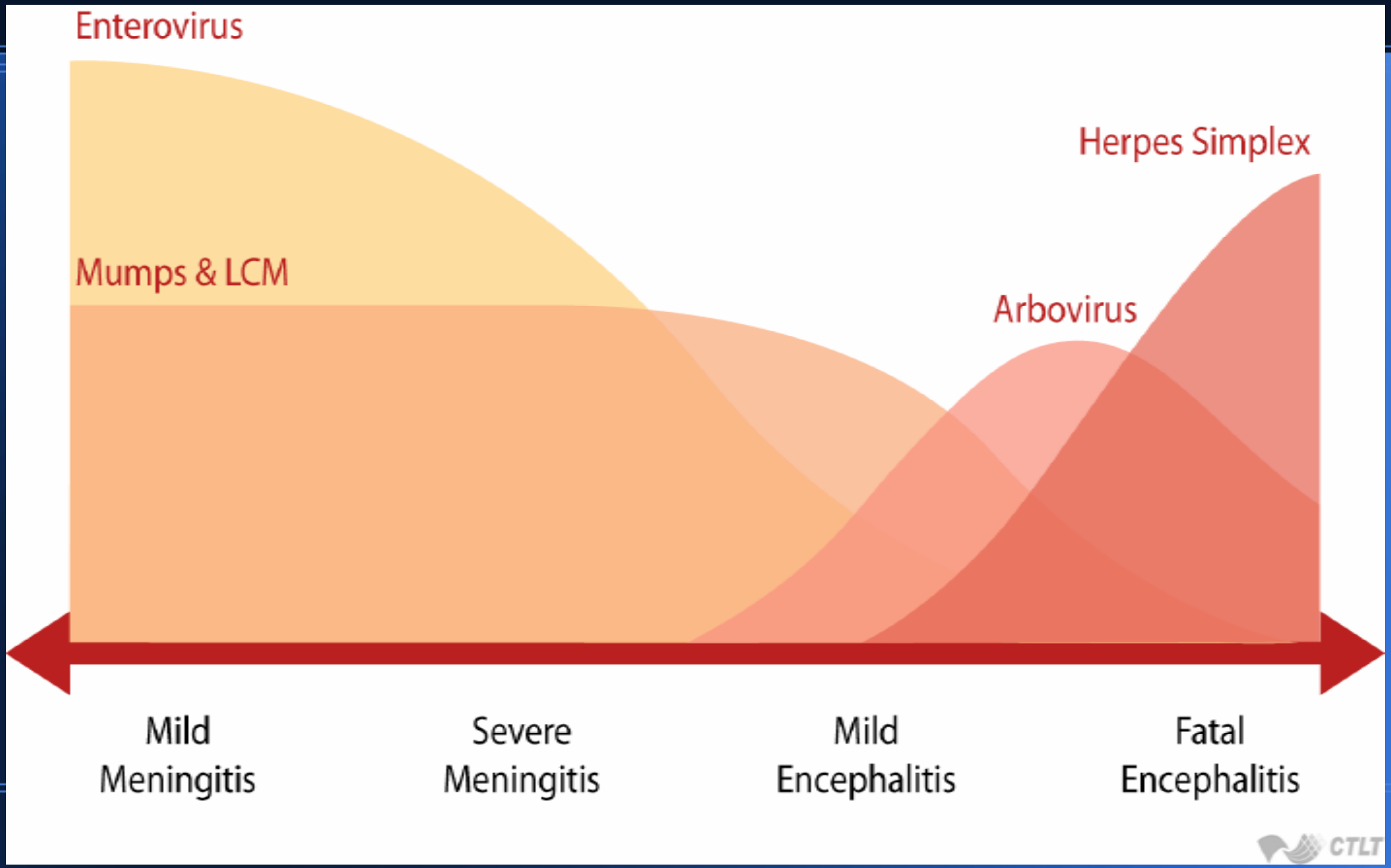
Pathogenesis

- In herpesvirus B infection and rabies --- the virus travels from the bite site along peripheral nerves to the CNS.
- In the arboviral encephalitides and in mumps and measles, viremia is the source of infection of the CNS . Encephalitis viruses are neurotropic and apparently replicate in neurons, thereby lysing them. In addition, later in infection there may be an immune inflammatory component to neuronal damage .

Histologically, there is perivascular infiltration by both mononuclear and polymorphonuclear cells.

Neuronal cells degenerate and are phagocytose

Viruses and Severity of Disease



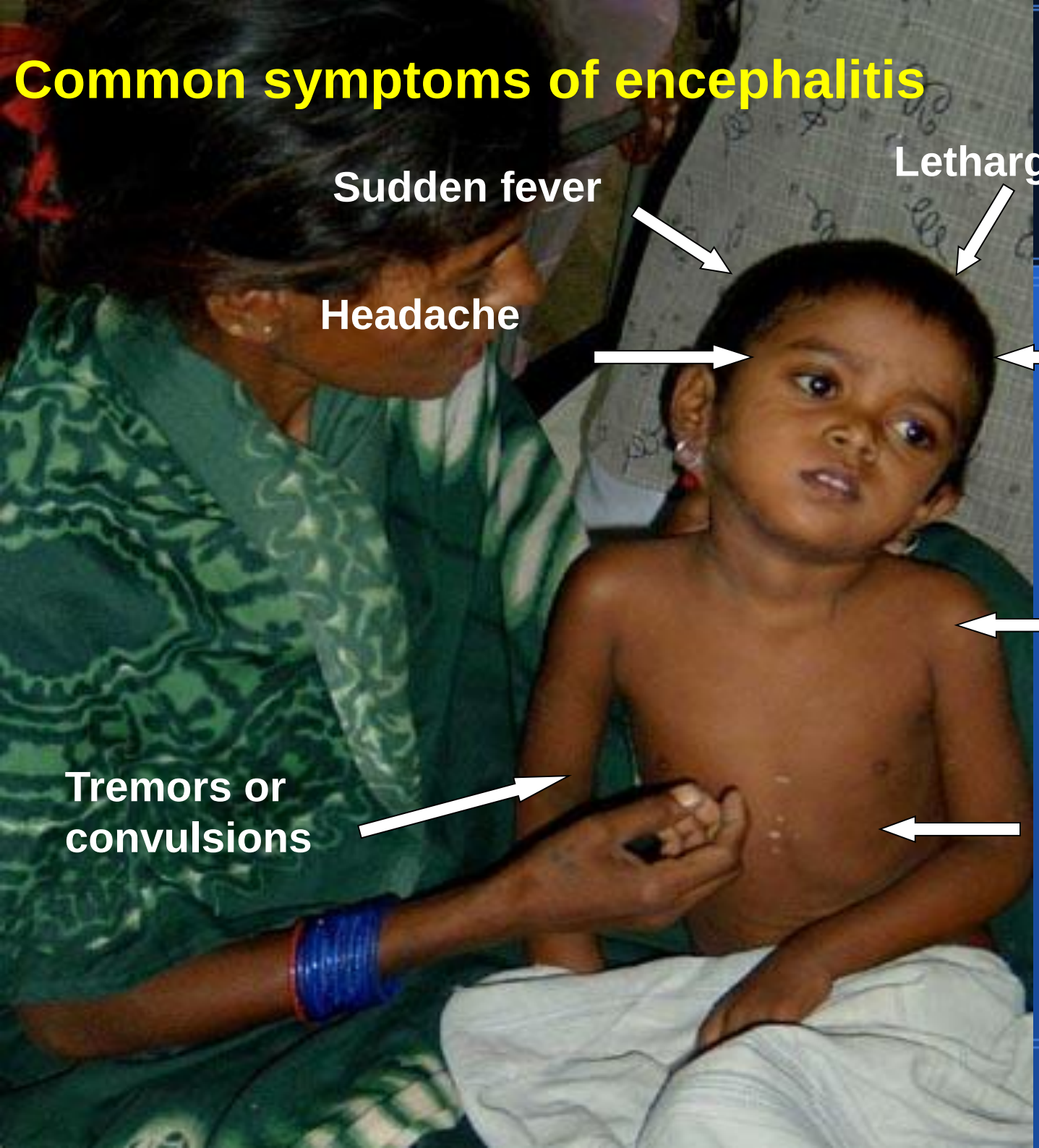
ENCEPHALITIS
Clinical

- Fever
- Malaise
- +/- Meningeal signs (headache, vomiting, stiff neck and back)
- Cerebral dysfunction: - altered consciousness
  - Personality changes
  - Seizure
  - Paresis
  - Cranial nerve abnormalities
- The clinical presentation of encephalitis is generally nonspecific:

Fever, headache, vomiting, occasionally accompanied by seizures, mental status chanes, and/or focal neurologic deficits

- Any patient presenting with fever and an abnormal neurologic exam should be evaluated closely for encephalitis!

Common symptoms of encephalitis



Sudden fever

Lethargy

Headache

Change in consciousness

Irritability or restlessness

Tremors or convulsions

Vomiting and diarrhea

Arboviruses

- Arboviruses or “arthropod-borne viruses” are the primary cause of encephalitis in many countries.
- Arthropods that transmit the viruses include mosquitoes and ticks.
- Common arboviruses include Japanese encephalitis, West Nile, and Dengue viruses.

Arboviral encephalitis

the incubation period may vary from several days to 2 weeks The vast majority (greater than 90%) of arboviral infections result in mild disease resembling an influenza-like illness.

the syndrome consists of fever, headache, malaise, and abnormal mental state progressing over several days to stupor and coma, accompanied by nuchal rigidity and seizures. After the first week, either flaccid or spastic paralysis may occur. Once signs and symptoms are present, they progress for 1 to 2 weeks, at which time the patient either dies or begins to show signs of recovery. Because of the damage to neurons, which are incapable of regeneration, there are often severe sequelae of infection.

St. Louis encephalitis---- severe disease in the elderly, especially in black persons and people with hypertension

Japanese B encephalitis and California virus encephalitis are most common in children.

West Nile virus

West Nile virus, a mosquito-borne flavivirus closely related to St. Louis encephalitis and Japanese encephalitis viruses, is endemic to Africa, the Middle East, and southwest Asia. The virus is transmitted between Culex mosquitos and wild birds, with incidental infections occurring in humans.

Most patients were at least 50 years old, had encephalitis, and presented with fever, weakness, nausea, vomiting, headache, and altered mental status. Twenty percent of the patients had an erythematous macular, papular, or morbilliform eruption involving some combination of the neck, trunk, and arms and legs. Decreased muscle strength and hyporeflexia were noted in a third of the cases. Older age was associated with a substantially higher risk of more severe neurologic disease.

CSF characteristics

Variable pleocytosis (10 to 2000 cells/mm^3) with mononuclear cells in herpes simplex encephalitis, a significant number of red blood cells may be found

Protein levels is usually increased in encephalomyelitis and in chronic infection (syphilis, Lyme disease)

Glucose level is within normal range

Culture and direct examination of CSF by Gram stain for bacteria, by acid-fast stain for mycobacteria and by India ink for Cryptococcus should be performed and may be diagnostic.

EEG is often abnormal

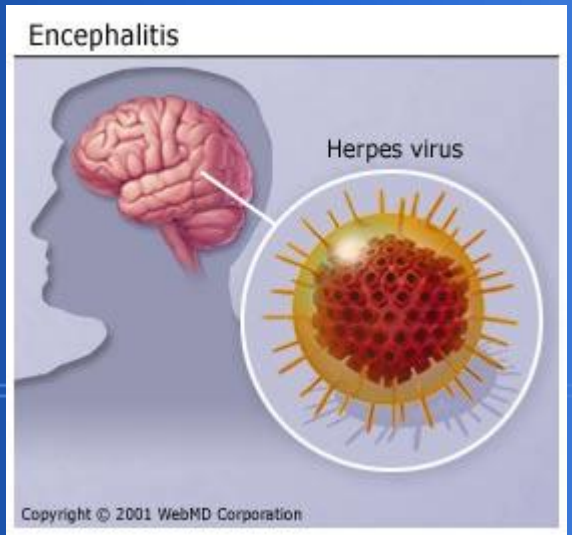
TREATMENT

Treatment

- antiviral drugs
  - HSV- acyclovir
  - CMV- gancyclovir, foscarnet
  - HIV - ARV
- Steroids
- Hyperosmolar agents
- Vigorous supportive therapy

The only form of viral encephalitis for which effective treatment exists is HSV encephalitis

early (before coma) institution of therapy with acyclovir. The drug is given intravenously at a dose of 30 mg/kg per day in three divided doses for at least 14 days.



Encephalitis vs. Meningitis

Encephalitis is distinguished from aseptic meningitis by the extent and severity of cerebral dysfunction, independent of signs of meningeal inflammation.

	Encephalitis	Viral Meningitis
Constitutional symptoms		
Fever	**Yes**	**Yes**
Headache, nausea, vomiting, lethargy	**Yes**	**Yes**
Photophobia, neck stiffness	**No**	**Yes**
Neurologic dysfunction		
Seizures	**Yes**	**Minimal**
Cranial nerve palsies, paralysis	**Yes**	**No**
Altered mental status (i.e. confusion, coma)	**Yes**	**Minimal**

Differential diagnosis of encephalitis

- Bacterial infection
- Other infections
- Meningitis, tuberculosis, brain abscess
- Cerebral malaria, Rickettsial, spirochetal, toxoplasmosis
- Intracranial hemorrhage or tumor
- Trauma
- Toxic ingestion
- Hypoglycemia
- Guillain-Barre syndrome

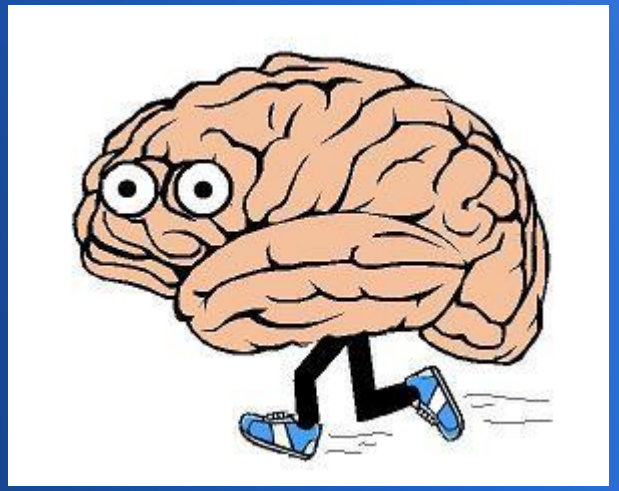
Prevention

- Vaccines have been developed against Japanese B, Venezuelan, and tick-borne encephalitis
- The best advice would be to take all measures available to prevent exposure to the mosquito or tick vectors.
- Mumps and measles vaccinations have decreased markedly the incidence of encephalitis due to these two diseases.
- The rabies vaccine is effective in preventing disease after the bite of an infected animal.

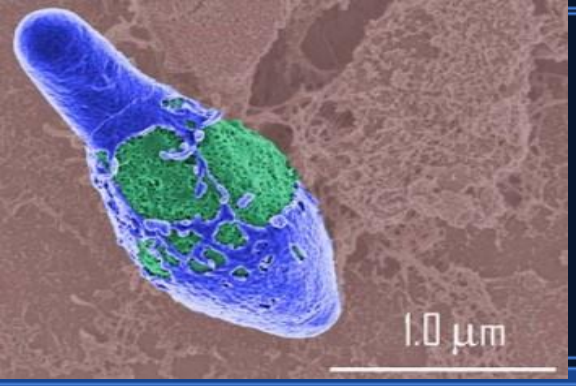
THANK YOU for your past support.

This event has been CANCELLED.

Please check back for future events.



Clostridium tetani



Clostridium tetani

Family -Clostridiaceae
Genus - Clostridium
Species - Clostridium tetani

Clostridium
Causing

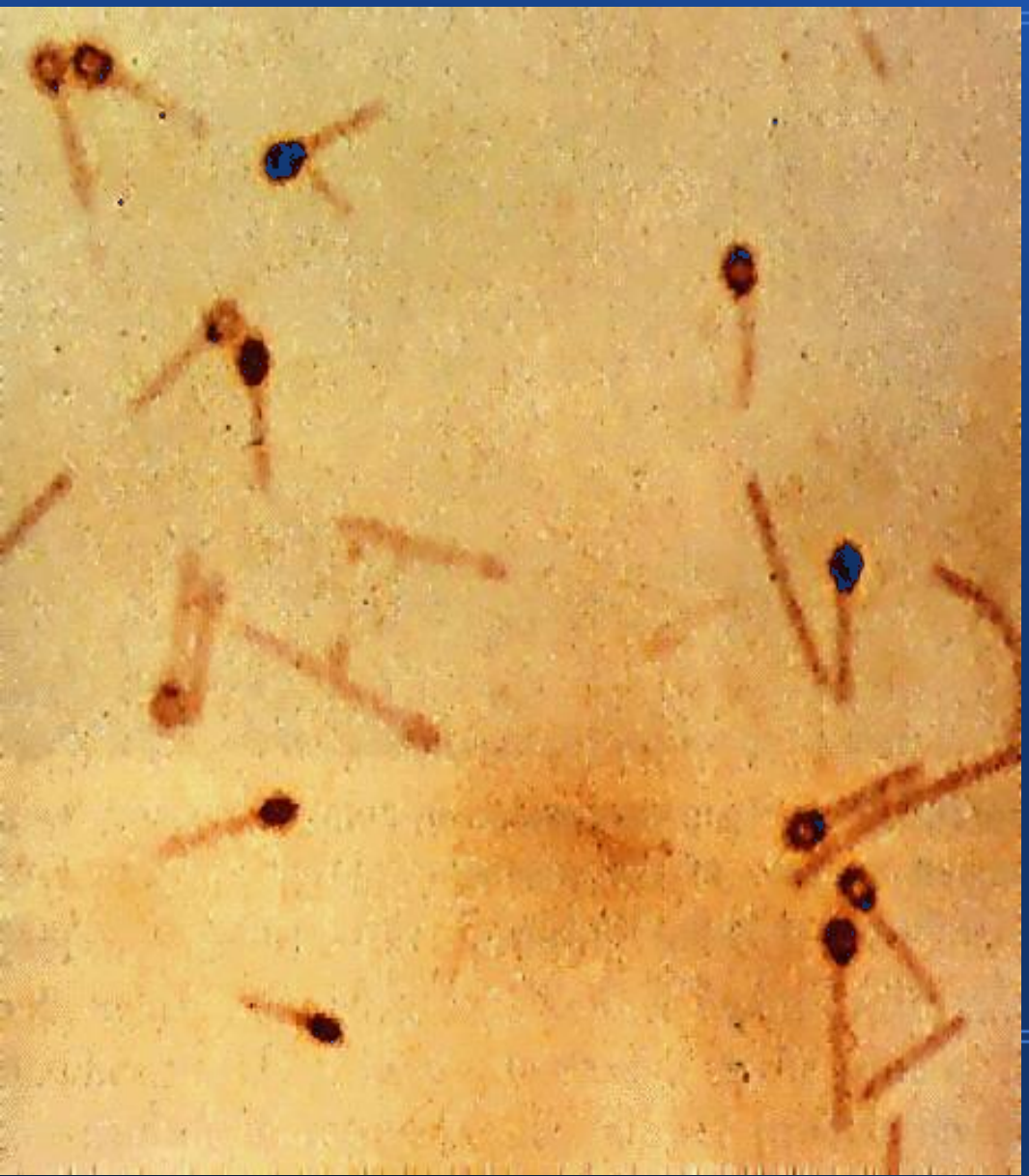
- **TETANUS** e.g. *Cl. tetani*
- **Gas gangrene**
- **BOTULISM** e.g. *Cl. botulinum*
- **Antibiotic associated diarrhea** e.g. *Cl. difficille*
- **Saccharolytic** e.g. *Cl. perfringens* &*Cl. septicum*
- **Proteolytic** e.g. *Cl. sporogenes*
- **Mixed:** *Cl. histolyticum*

Tetanus toxin

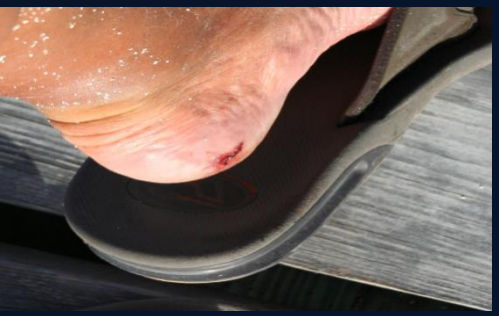
- Two toxins, TETANOSPASMIN (tetanus toxin) and Tetanolysin, are produced by C. Tetani.
- TETANOSPASMIN- is encoded on a plasmid, witch is present in all toxigenic strains. Per weight, tetanospasmin is one of the most potent toxins known, being surpassed in potency only by botulinum toxin. The estimated minimum lethal dose is 2.5 nanograms per kilogram of body weight
- Tetanolysin – is of uncertain importance in the pathogenesis of tetanus

Clostridium tetani is not a tissue-invasive organism, it cause illness through the effect of a single toxin TETANOSPASMIN.

Clostridium tetani Gram stain



- Gram positive, straight, slender rod with rounded ends, nonencapsulated
- All species form endospore with “drumstick” or “tennis racket” appearance
- Fermentative
- Obligate anaerobe
- Motile by peritrichous flagella
- Spores are highly resistant to adverse conditions



Clostridium tetani Epidemiology

- Reservoir:

Spores of C. tetani are found in soil, house dust, animal intestines, and human feces------> can survive for a long time in environment---100 yrs possibly!
Spores usually enter through accidental puncture wounds, burns, umbilical stumps, frostbite, and crushed body parts. Anaerobic environment is required for vegetative cells to grow and release toxin.

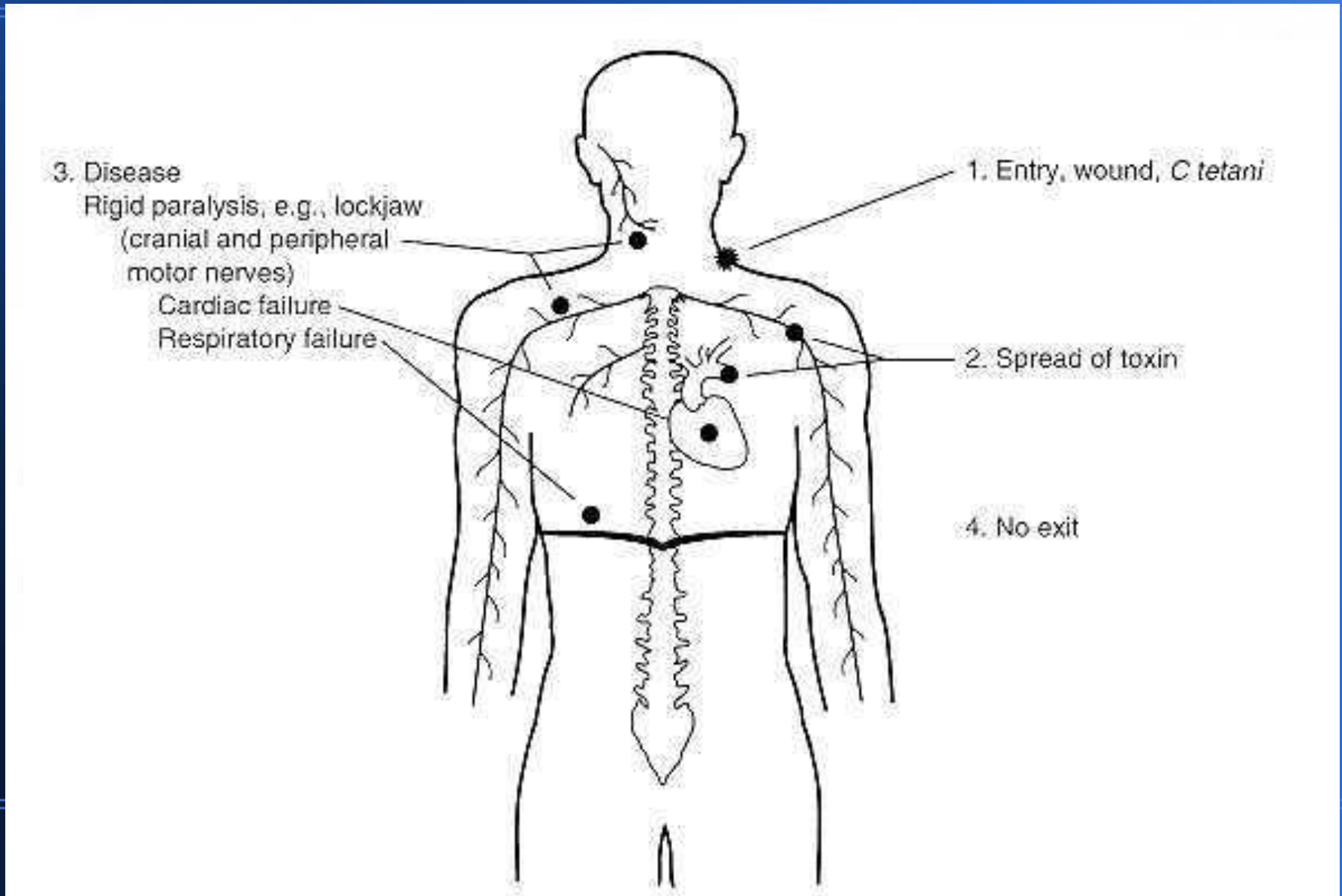
- Communicability

  - Tetanus is not contagious from person to person.
  - It is the only vaccine-preventable disease that is infectious but not contagious.

- Temporal pattern: Peak in winter and summer season

- Immunity from tetanus decreases with advancing age

Tetanic Infection



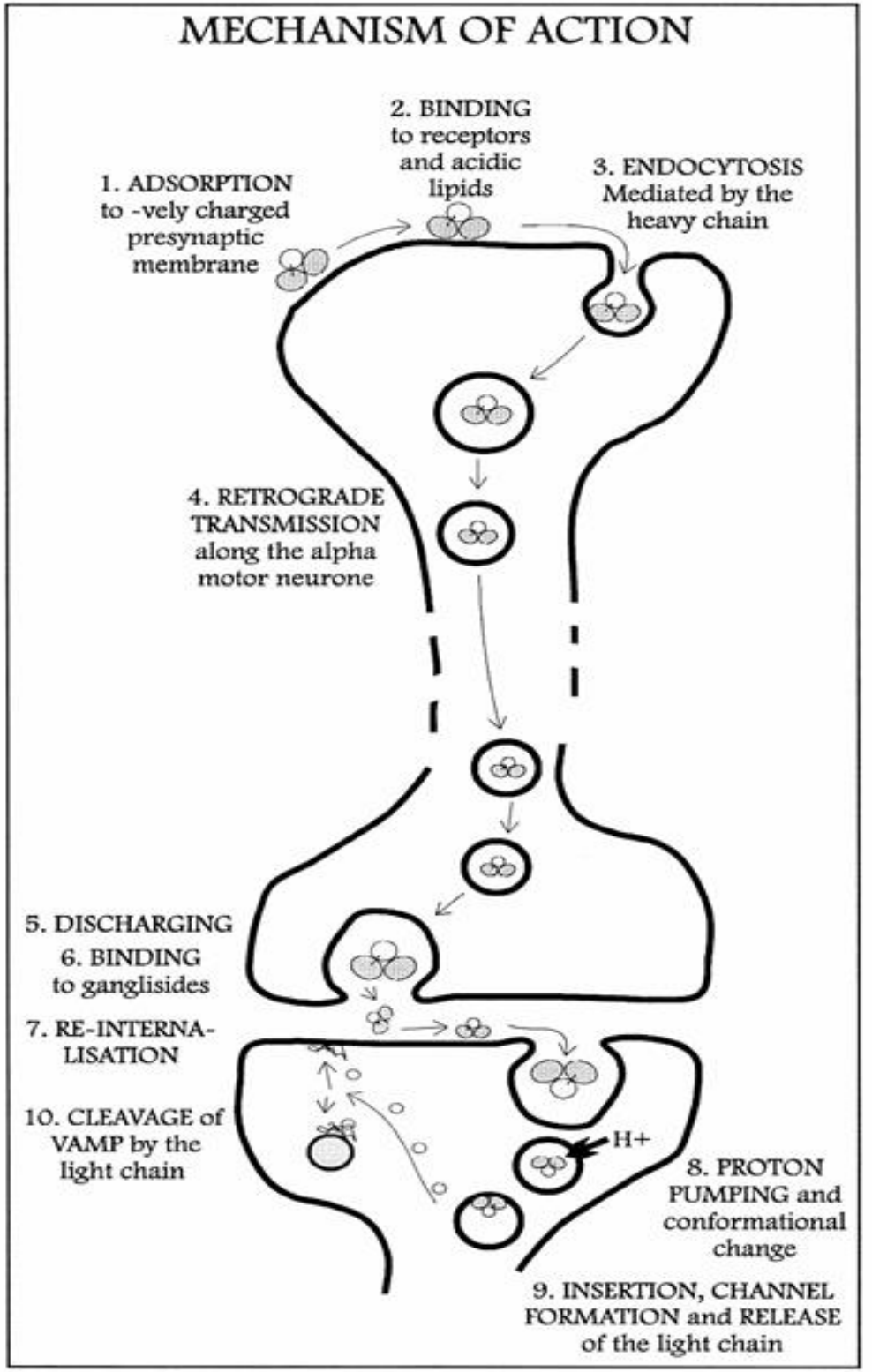
Pathogenesis and Immunity

Tetanospasmin is responsible for clinical manifestations of tetanus.

Contamination of devitalized tissue (wound, burn, injury, umbilical stump, surgical suture) with the spores------> germination of the spores------> release of tetanospasmin ----> the toxin reaches CNS by retrograde axonal transport or via the bloodstream-------> the toxin is fixed to gangliosides in spinal cord or brainstem and exerts its actions.

The Course of Tetanus

Once in the spinal cord, tetanospasmin is released from the motor neuron.The neurons, which release gamma-aminobutyric acid (GABA) and glycine, the major inhibitory neurotransmitters, are particularly sensitive to tetanospasmin, leading to failure of inhibition of motor reflex responses to sensory stimulation.



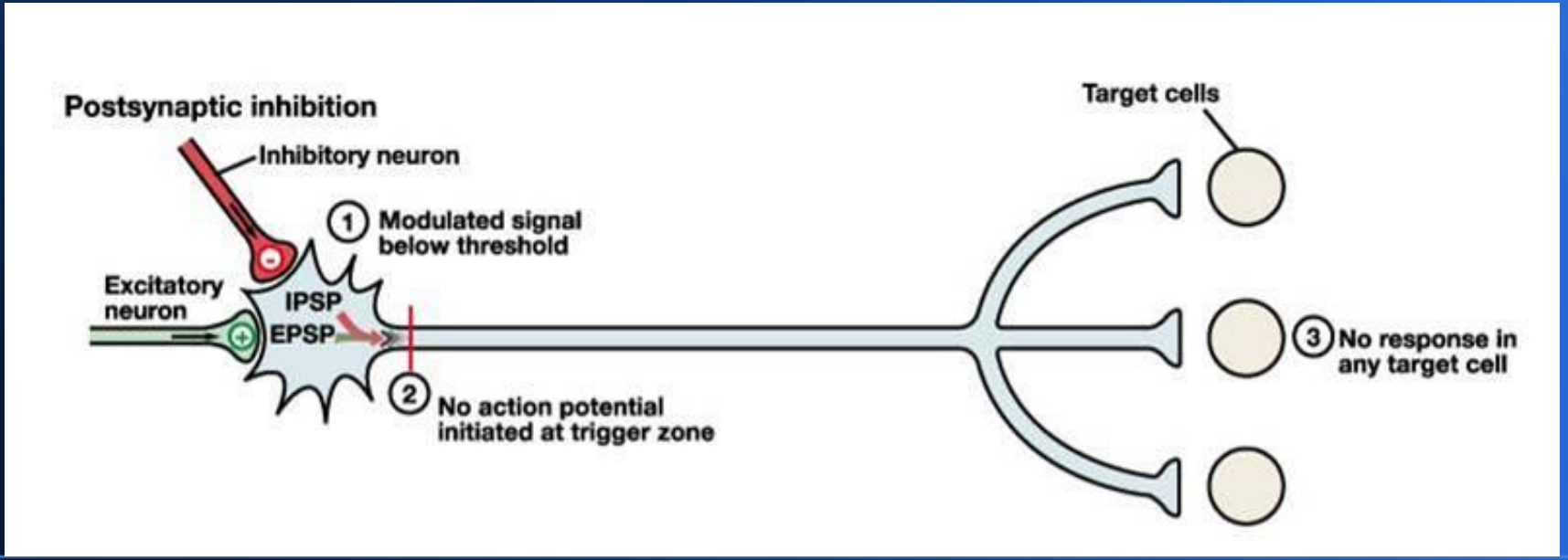
Analogy

- Think of the Inhibitory pathway as your parents, and the Excitatory pathway as your friends.
  - If a group of your parents' friends take them away for a weekend out, the friends are like **TETANUS** because they are removing your inhibitory control.
  - When your friends come over for the party you're throwing your excitatory pathway is uncontrolled because your inhibitory pathway has been incapacitated.
  - This results in muscle spasms, and potentially death.

The Course of Tetanus

This results in generalized contractions of the agonist and antagonist musculature characteristic of a tetanic spasm.

Patients experience muscle spasms that begin in the jaw and may eventually effect the entire body. When the extremities become involve the arms and legs may go into painful and rigid spasms.



Sequence of events

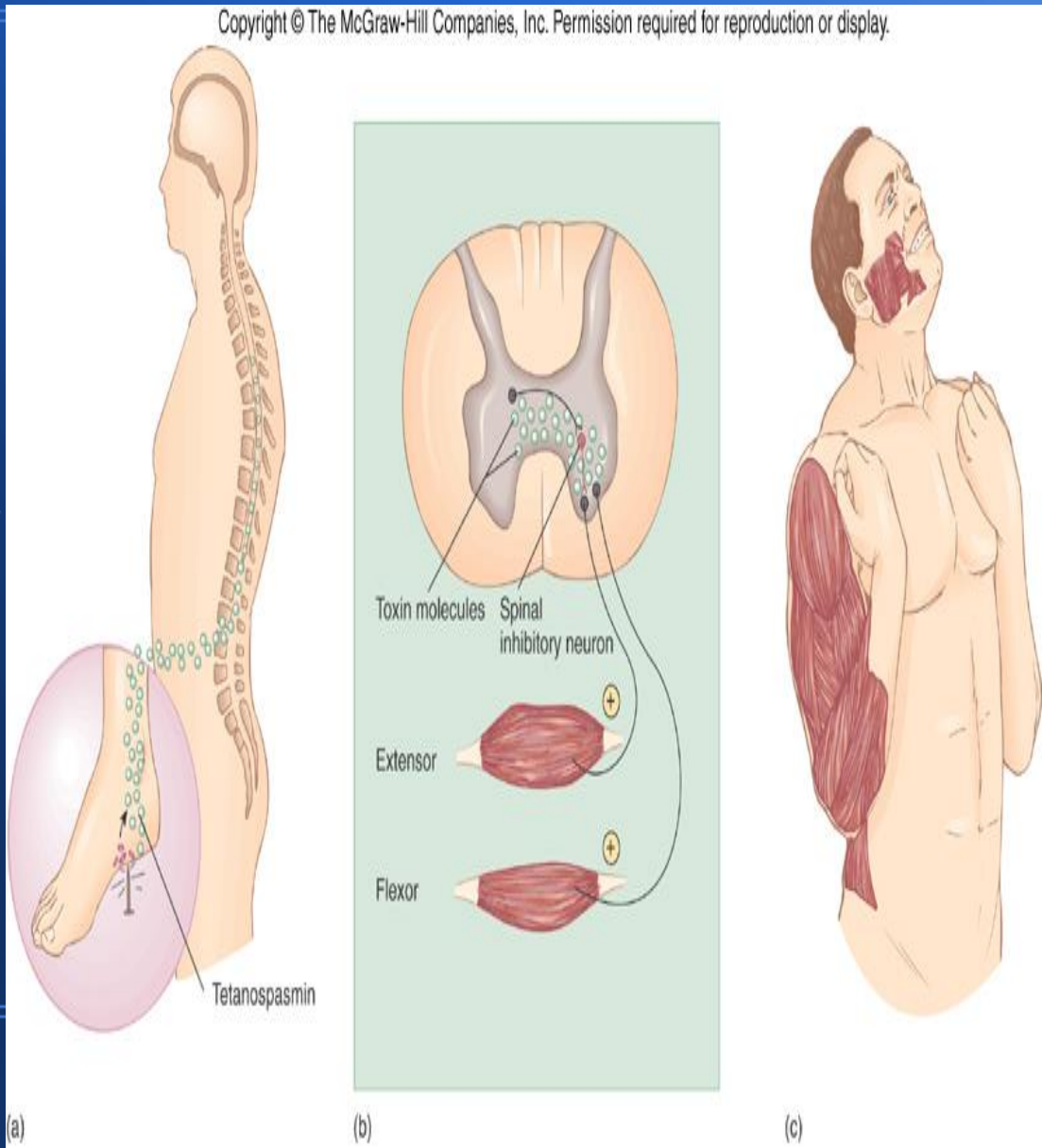
Lock Jaw

Stiff Neck

Difficulty Swallowing

Muscle Rigidity

Spasms



Clinical

The incubation period ranges from 3 to 21 days

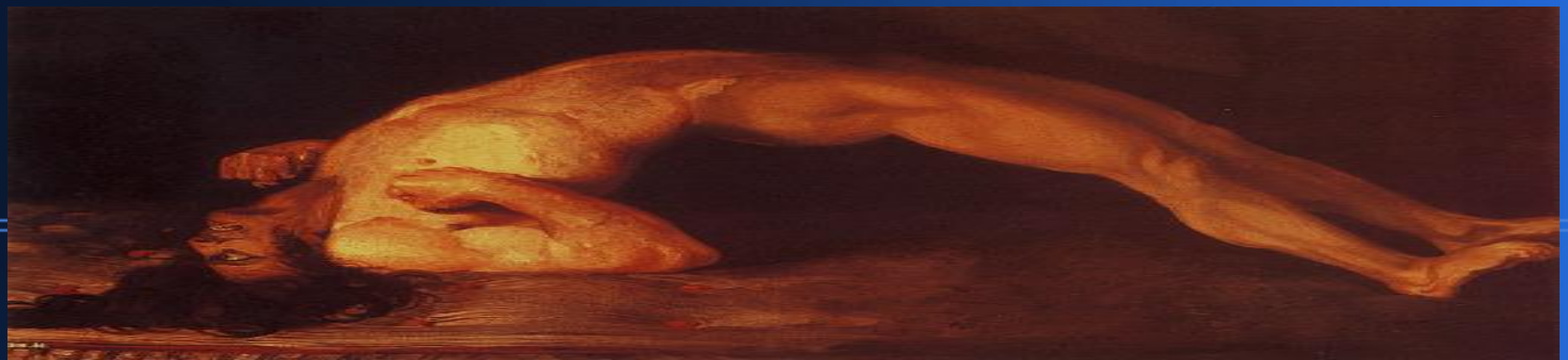
GENERALIZED TETANUS

- Trismus – masseter muscle spasm or lockjaw is the presenting symptom in 50% of cases
- Headache, irritability and restlessness – early symptoms followed by stiffness, difficulty chewing, dyspahgia and neck muscle spasm

Dysphagia occurs in moderately severe tetanus due to pharyngeal muscle spasms, and onset is usually insidious over several days.

Sustained contraction of facial musculature produces a sneering grin expression known as risus sardonicus

- When the paralysis extends to abdominal, lumbar, hip, thigh muscles, the patient may assume an arched posture of extreme hyperextension - opisthotonos (head and heel are bent backward, the body bowed forward, only the back of the head and heels touching the supporting surface)



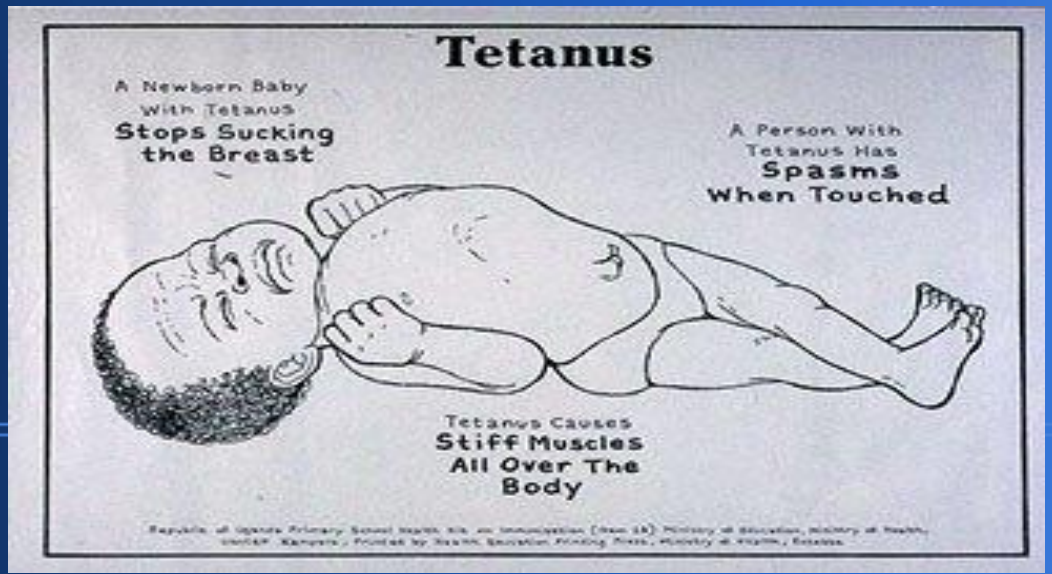
GENERALIZED TETANUS

- Laryngeal and respiratory muscle spasm ----> airway obstruction ---->asphyxiation
- 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
- Because tetanus toxin does not affect sensory nerves or cortical function, the pacient unfortunately remains conscious, in extreme pain.
- Fever – because of the substantial metabolic energy consumed by spastic muscles.
- Notable autonomic effects: tachycardia, arrythmias, labile hypertension, diaphoresis and cutaneous vasoconstriction.
- The tetanic paralysis becomes more severe in the 1st week after the onset, stabilizes in the 2nd wk, and ameliorates gradually over 1-4 weeks.

Neonatal tetanus

Worldwide risk factors for neonatal tetanus are as follows:

- Unvaccinated mother, home delivery, and unhygienic cutting of the umbilical cord increase susceptibility to tetanus.
- 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, clarified butter) are risk factors for neonates.
- Manifests within the 3- 12 day of birth as a progressive difficulty in feeding (sucking and swallowing).
- Paralysis or diminished movement, stiffness to the touch, and spasms ± opisthotonos.
- The mortality rate exceeds 70%



Localized tetanus

- Results in painful spasms of the muscles adjacent to the wound site and may precede generalized tetanus.
- Cephalic tetanus – is a rare form of localized tetanus involving the bulbar musculature that occurs with wounds of foreign bodies in the head, nostrils, or face. It also occurs in association with chronic otitis media.
- Retracted eyelids
- Deviated gaze
- Trismus
- Risus sardonicus
- Spastic paralysis of tongue and pharyngeal muscle

Complications

Death Generally Results from Complications which include:

- Laryngospasm
- Spasm of the muscles of respiration which may lead to interference with breathing.
- Fractures of the spine or long bones from sustained contractions and convulsions.
- Hyperactivity of the autonomic nervous system may lead to hypertension and/or an abnormal heart rhythm.

Tetanus is most likely to be fatal for persons 60 years and older and those who are unvaccinated.

Diagnosis of Tetanus

- Tetanus is suspected upon exposure to a bite or puncture wound
- Because C. tetani exhibits such exquisite sensitivity to oxygen, it is very difficult to recover and/or grow from clinical specimens
- As a result, diagnosis is made on the basis of clinical findings and history

Laboratory Diagnosis of Tetanus

- The diagnosis of tetanus depends primarily upon the clinical manifestation of tetanus including muscle spasm and rigidity.

- **Specimen:** Wound exudates using capillary tube

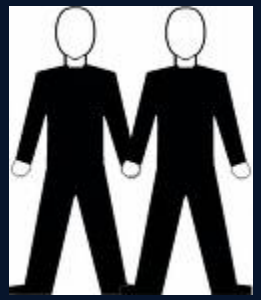
- **Culture:**

On blood agar and incubated anaerobically

Growth appears as a fine spreading film.

- Gram stain is a good method for identifying *Clostridium*

Cl. tetani is Gram positive rod motile with a round terminal spore giving a drumstick appearance



Differential diagnosis

- Meningitis/ encephalitis – CSF
- Rabies – hydrophobia, marked dysphagia, predominantly clonic seizures, CSF -pleocytosis
- Strychnine poisoning – tonic muscle spasms and generalized seizure activity, general relaxation ussualy occurs between spasms
- Hypocalcemia – tetany, laryngeal and carpopedal spasms, but trismus is absent
- Epileptic seizure – EEG
- Narcotic withdrawal
- Dystonic drug reaction

Three Objectives of Management of Tetanus

1) To provide supportive care until the tetanospasmin that is fixed in tissue has been metabolized

1) To neutralize circulating toxin

1) To remove the source of tetanospasmin.

Treatment

- surgical wound excision and debridement to remove the foreign body or devitalized tissue that created anaerobic growth condition
- passive immunity (human tetanus immunoglobulin) – as soon as possible to neutralize toxin that diffuses from the wound into the circulation before toxin can bind at distant muscle groups
- antibiotics (Penicillin G 100.000 ui/kg/day Q 4-6 hr iv for 10-14 days or Metronidazole 500 mg q 8 hr iv - clostridiocidal action)
- generalized tetanus needs muscle relaxants Diazepam 0,1-0,2 mg/kg 3-6 hr iv
- preventative active immunization with tetanus toxoid (toxin detoxified with formalin) DPT vaccine

Tetanus Prophylaxis and Wound Management

Tetanus Prophylaxis and Wound Management

	Clean, minor wounds		All other wounds*	
Vaccination history	*Td†*	*TIG*	*Td†*	*TIG*
Unknown or < 3 doses	Yes	No	Yes	Yes
≥3 doses	Only if last dose >10 yrs	No	Only if last dose >5 yrs	No

*Such as, but not limited to, wounds contaminated with dirt, feces, soil, and saliva; puncture wounds; avulsions; any wounds resulting from missiles, crushing, burns, and frostbite.

†If patient is <7 years of age, give DTaP. If patient is between 7 and 10 years of age, or ≥65 years, give Td. Otherwise (i.e., between 11 and 64 years of age) give Tdap.

Source: Adapted from VPD Surveillance Manual, 4th Edition, 2008. Tetanus: Chapter 16. Available at: *www.cdc.gov/vaccines/pubs/surv-manual/default.htm*.

Prognosis

Favorable

- Long incubation period
- Absence of fever
- Localized disease

Unfavorable

- A week or less between the injury and the onset of trismus
- 3 days or less between trismus and onset of generalized tetanic spasms

Tetanus Prophylaxis

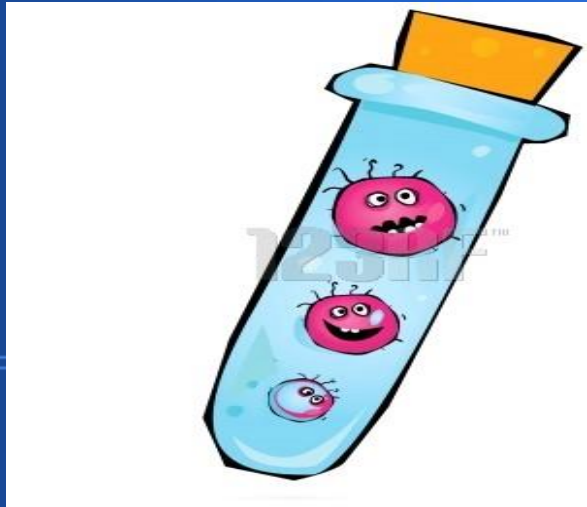
Dose	Age	Minimum Interval
Dose 1	2 months	---
Dose 2	4 months	4 wks
Dose 3	6 months	4 wks
Dose 4	15-18 months	6 months
Dose 5	Prior to school	

Tetanus Prophylaxis

- DTaP (diphtheria, tetanus, acellular pertussis) - has greatly reduced the incidence of neurological adverse effects observed with the earlier "whole-cell" pertussis vaccine : seizures and hypotonic episodes immunization.
- DTaP immunizations are routinely given in five doses before a child's sixth birthday
- This regimen provides protection from tetanus for about 10 years, and every 10 years thereafter, a booster shot of tetanus vaccine is recommended.

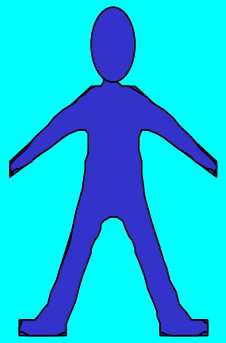

Thank you!

Laboratory Diagnosis of Bacterial Diseases

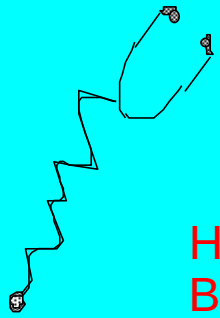


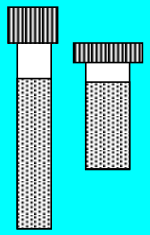
Diagnosis in Infection Diseases

Patient



Clinical diagnosis

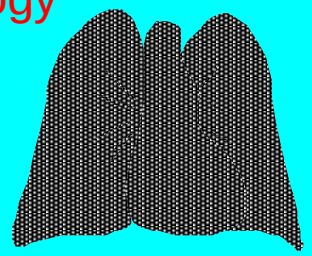




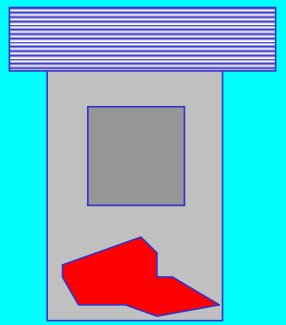
Haematology
Biochemistry

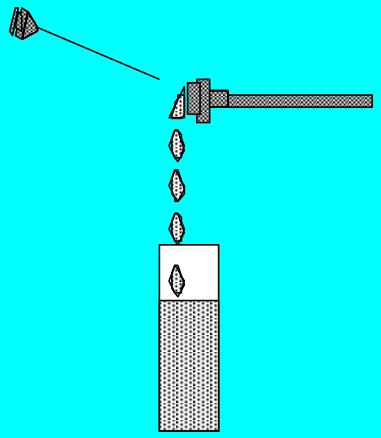
Non-microbiological investigations

Radiology



Sample





Take the correct specimen

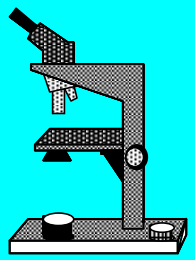
Take the specimen correctly

Label & package the specimen up correctly

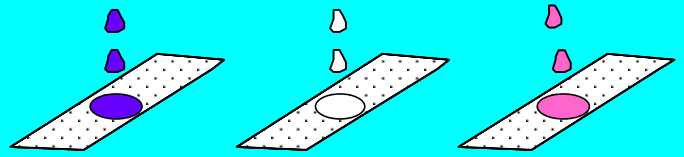
Appropriate transport & storage of specimen

Diagnosis of Bacterial Infection

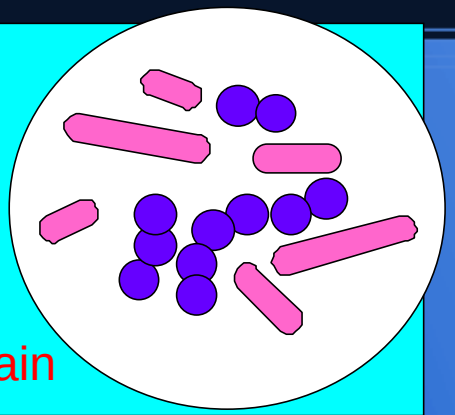
microscopy



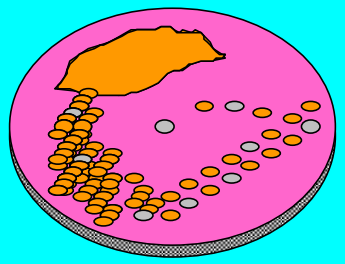
unstained or stained with e.g. Gram stain

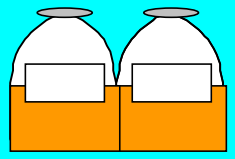


Stain Decolorise Counterstain



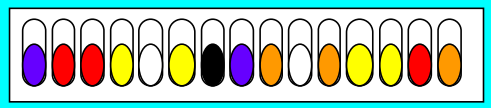
culture



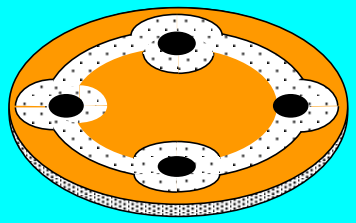


on plates or in broth

identification by biochemical or serological tests on pure growth from single colony

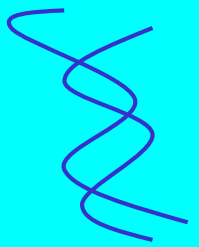


sensitivities



by disc diffusion methods, breakpoints or MICs

Serodiagnosis



DNA technologies

Collecting the specimen correctly

- Take an mid-stream urine
  - avoids contamination with perineal flora
- CSF
  - Avoid contamination
  - Avoid bloody tap
- Throat swab
  - Make the patient gag!
- Sputum , not saliva
- Blood cultures
  - Avoid contamination with skin organisms

Getting the specimen to the lab

- Problems in delay or inappropriate storage• delay in dignosis & treatment
  - pathogens die
  - contaminants overgrow
- Blood cultures directly into incubator
  - not refigerator!
- CSF straight to lab
- Don't put an entire surgical specimen into formalin!
  - Send a portion to microbiology in a sterile container

Microscopy

The **Gram stain** is a rapid, inexpensive method for demonstrating the presence of

- bacteria
- fungi
- inflammatory cells

A preliminary assessment of the etiologic agent can be made by

- morphology of the microorganisms (cocci vs rods)
- their color (gram-positive microorganisms stain blue, gram-negative stain red).

The presence of inflammatory and epithelial cells can be used to gauge the quality of certain specimens. For example, ≥10 epithelial cells per low-power field in a sputum sample strongly suggest contamination from oral secretions.

The Gram stain is an insensitive technique, requiring 10^4-10^5 microorganisms/mL for detection.

Isolation and identification

Most medically important bacteria can be cultured on nutrient-rich media such as

- blood
- agar
- chocolate agar.

Liquid broth media are used for blood cultures and to enhance growth of small numbers of organisms in other clinical specimens.

Sabouraud dextrose agar (with antibiotics to inhibit bacterial growth) is used to culture most fungi.

Others: *Bartonella, Bordetella pertussis, Brucella, Francisella, Legionella,* and mycobacteria, and certain fungal pathogens such as *Malassezia furfur, Mycoplasma,* and *Chlamydia* require specialized growth media or incubation conditions.

After isolation in culture, microbial identity can be confirmed by a series of biochemical tests, by the ability of the organism to grow in the presence of certain substances that inhibit growth of other microorganisms (selective antibiotics, salt, bile), or by antigen detection.

Blood Culture

Several different blood culture systems are available. Most use 50-100 mL bottles containing broth that enhances the growth of bacteria and fungi (mainly yeast). Bottles with smaller volumes are also available specifically for pediatric use. Media containing resins are often used to adsorb antibiotics that may be present in a patient's blood and to improve microbial detection. Most laboratories use automated systems that greatly reduce the time to microbial detection; >80% of all cultures containing pathogens become positive within 24 hr of incubation.

Proper skin disinfection before blood collection is essential.

- Povidone-iodine may be used, but this agent must be allowed to dry completely.
- Alcohol is rapidly bactericidal and is a suitable alternative agent.

Iodine is effective but must be wiped off with alcohol to avoid skin reactions.

For patients with suspected bacteremia or fungemia, 2 or 3 separate blood cultures are preferred. Whenever possible, at least 2-3 mL of blood should be obtained for culture before administration of antibiotics.

Blood should also be cultured anaerobically for patients at increased risk for anaerobic sepsis, such as children who are immunocompromised or who have head and neck or abdominal infections. Detection of fungi can be aided by lysis-centrifugation techniques.

Growth medium

Gram stain	Stains bacteria, fungi, leukocytes, and epithelial cells
Potassium hydroxide (KOH)	A 10% solution dissolves cellular and organic debris and facilitates detection of fungal elements.
Calcofluor white stain	Nonspecific fluorochrome that binds to cellulose and chitin in fungal cell walls. Can be combined with 10% KOH to dissolve cellular material.
Ziehl-Neelsen and Kinyoun stains	Acid-fast stains, using basic carbolfuchsin, followed by acid-alcohol decolorization and methylene blue counterstaining. Acid-fast organisms (e.g., *Mycobacterium, Cryptosporidium,* and *Cyclospora*) resist decolorization and stain pink. A weaker decolorizing agent is used for partially acid-fast organisms (e.g., *Nocardia*).
Acridine orange stain	Fluorescent dye that intercalates into DNA. At acid pH, bacteria and fungi stain orange, and background cellular material green.
Auramine-rhodamine stain	Acid-fast stain using fluorochromes that bind to mycolic acid in mycobacterial cell walls, and resist acid-alcohol decolorization. Acid-fast organisms stain orange-yellow against a black background.
India ink stain	Detects *Cryptococcus neoformans,* an encapsulated yeast, by excluding ink particles from the polysaccharide capsule. (Direct testing of specimens for cryptococcal antigen is much more sensitive than India ink preparations.)

Methenamine silver stain	Stains fungal elements, *Pneumocystis* cysts in tissues. Primarily performed in surgical pathology laboratories

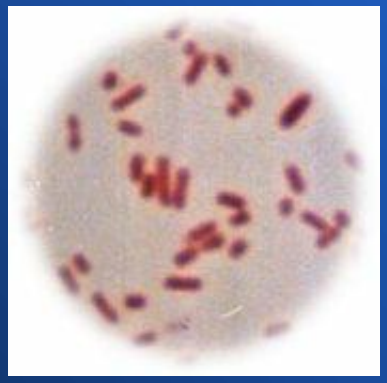
Lugol's iodine stain	Added to wet preparations of fecal specimens for ova and parasites to enhance contrast of the internal structures (nuclei, glycogen vacuoles)
Wright and Giemsa stains	Primarily for detecting blood parasites (*Plasmodium, Babesia,* and *Leishmania*), fungi in tissues (yeasts, *Histoplasma*)

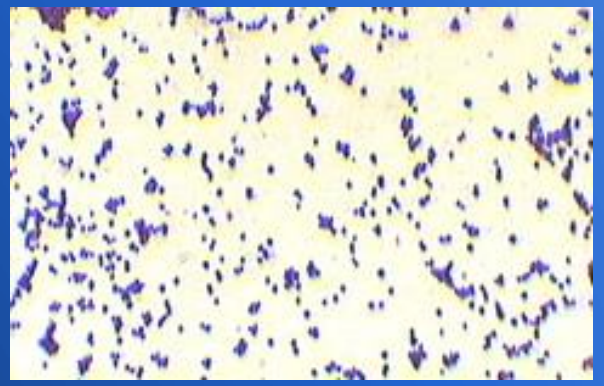
Trichrome stain	Stains stool specimens for identification of protozoa body
Direct fluorescent-antibody stain	Used for direct detection of a variety of organisms in clinical specimens by using specific fluorescein-labeled antibodies, e.g., *Bordetella pertussis, Legionella, Chlamydia trachomatis, Pneumocystis carinii,* many viruses.

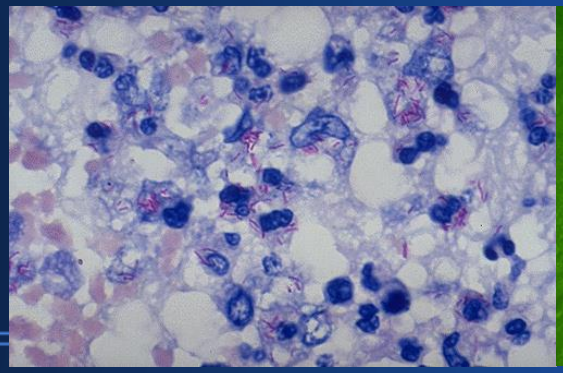
Microscopy

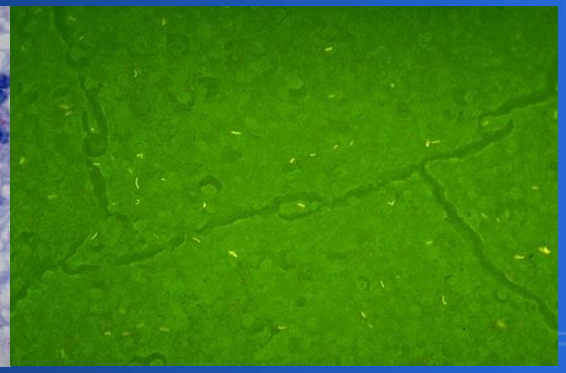
Stained preparations

- Gram-stain
- Acid-fast stain
  - Ziehl-Nielsen
- Fluorescence
  - Direct, e.g. auramine
  - Immunofluorescence









CSF culture

Urine culture

Urine for culture and colony count can be obtained by collecting

- clean-voided midstream specimens,
- by catheterization,
- by suprapubic aspiration.

Urine samples collected by placing bags on the perineum are unacceptable for culture because of frequent contamination that renders the results uninterpretable.

Urine collected by catheterization reflects infection if there are $\geq 10^3$ organisms/mL.

Clean-voided urine is considered abnormal if $\geq 10^5$ organisms/mL

However, lower counts are sometimes found in urinary tract infections in adolescent girls and young women, especially those with bacterial urethritis, or in patients with fungal infections. A Gram stain of unspun urine with ≥1 bacterium per oil immersion field correlates well with the presence of $\geq 10^5$ organisms/mL

Genital Culture

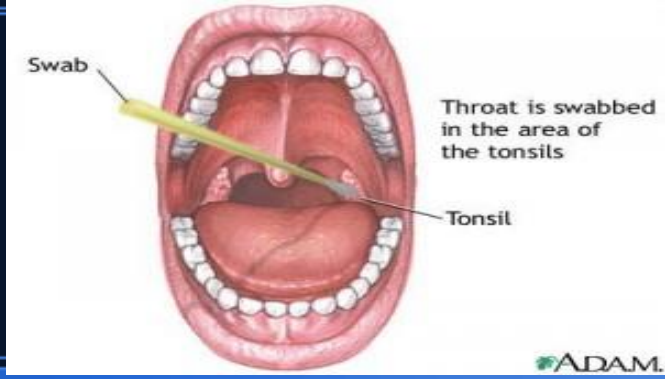
Specimens from the genital tract include urethral, cervical, and anorectal swabs.

Neisseria gonorrhoeae organisms are fragile, and rapid inoculation at the bedside onto Thayer-Martin medium (warmed to room temperature) or 1 of its modifications is crucial.

Chlamydia trachomatis culture are obtained by cotton-tipped, aluminum-shafted urethral swabs. Endocervical specimens, using swabs with aluminum or plastic shafts, should be collected by rubbing the swab vigorously against the endocervical wall to obtain as much cellular material as possible.

C. trachomatis is cultured by inoculation into cell culture systems, followed by immunofluorescent staining with monoclonal antibody against the organism

Throat and Respiratory Cultures



Obtaining a throat swab for culture is the most reliable method of diagnosing group A streptococcal pharyngitis and tonsillitis. Vigorous swabbing of the tonsillar area and posterior pharynx is necessary for maximum detection. Even then, a single swab detects only approximately 90% of infections.

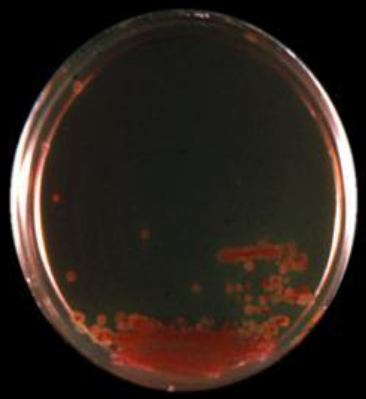
B. pertussis cultures are obtained by aspiration or a Dacron or flexible aluminum wire calcium alginate swab (Calgiswab) of the nasopharynx and inoculation onto special charcoal-blood (Regan-Lowe) or Bordet-Gengou media.

The cause of lower respiratory tract disease in children is not easy to confirm microbiologically because of difficulty in obtaining adequate sputum specimens and lack of correlation between upper respiratory tract flora and organisms causing lower respiratory tract disease.

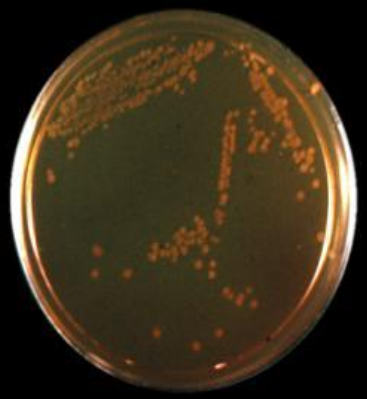
- endotracheal aspirates from intubated patients may be useful if the Gram stain shows abundant neutrophils and bacteria, although pathogens recovered from such specimens may still reflect only contamination from the endotracheal tube or upper airway.
- bronchoalveolar lavage fluid or bronchial brush specimens may be valuable for distinguishing upper respiratory tract contamination from lower tract disease in special circumstances.
- pulmonary tuberculosis in young children is best made by culture of early morning gastric aspirates, obtained on 3 successive days. Acid-fast stains of gastric aspirates

Stool Culture

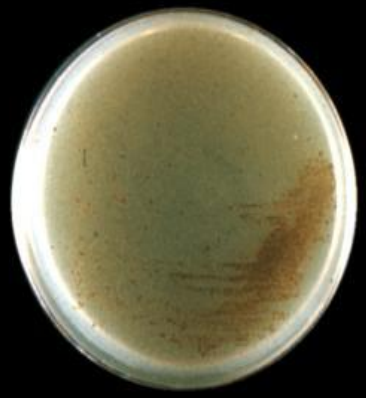
Shigella sp., Escherichia sp., and Proteus sp.



MacConkey Agar



Shigella-Salmonella Agar



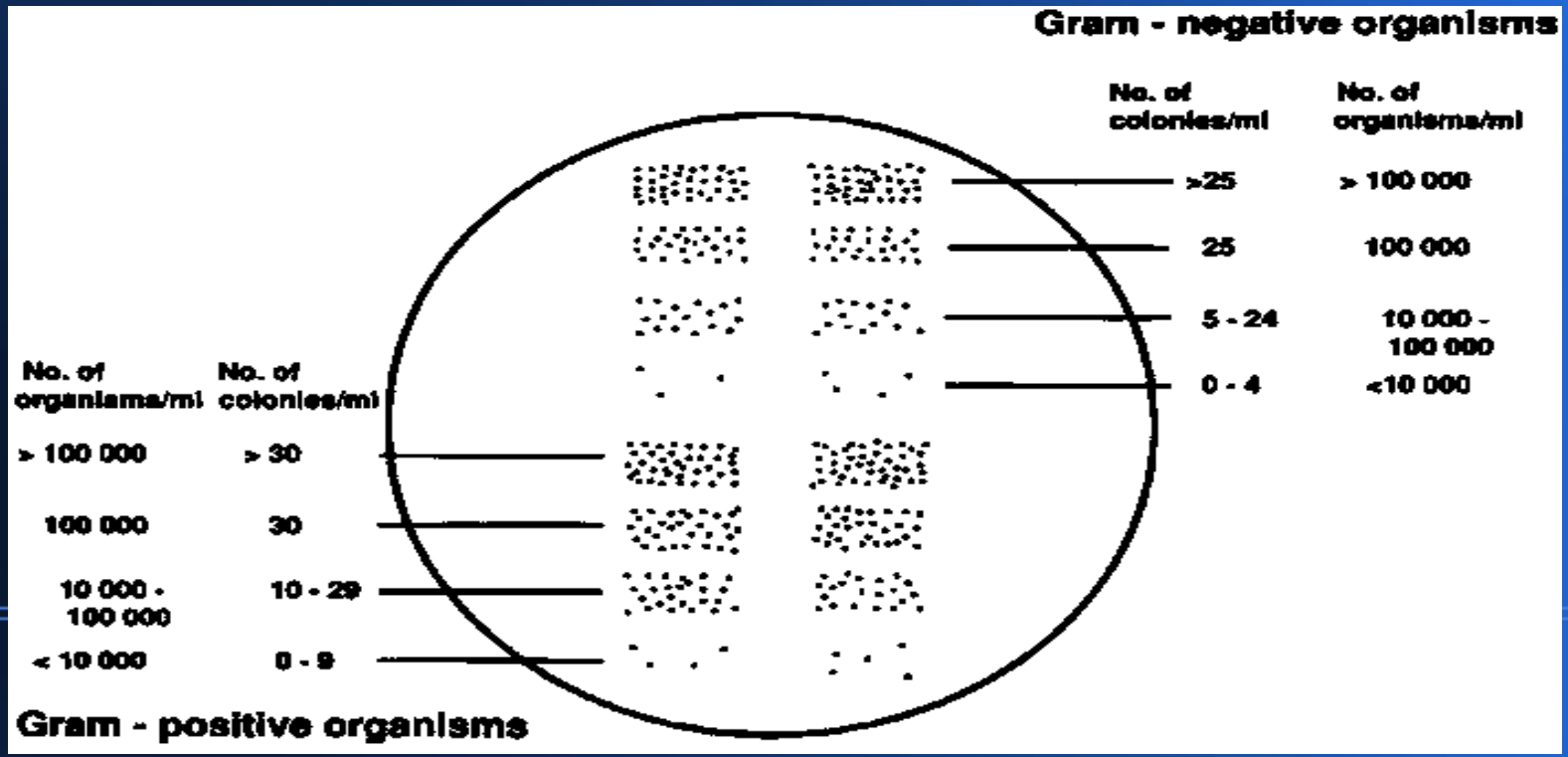
Bismuth Sulfite Agar

Brilliant Green Agar

Culture of Other Fluids and Tissues

Abscesses, wounds, pleural fluid, peritoneal fluid, joint fluid, and other purulent fluids are cultured onto routine solid agar and broth media.

Anaerobic organisms are involved in many abdominal and wound abscesses. These specimens should be collected and transported rapidly under anaerobic conditions, preferably in anaerobic transport tubes

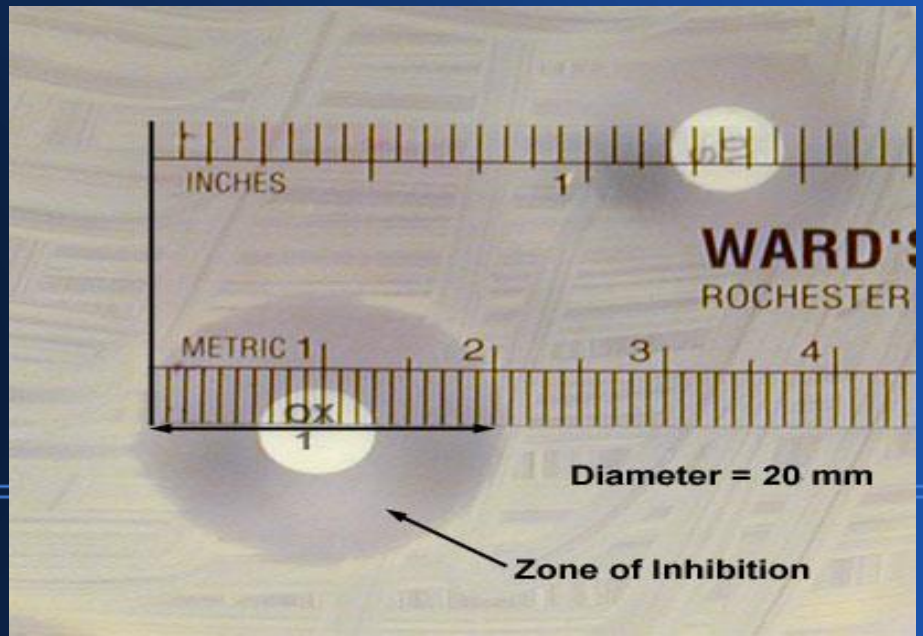


Antimicrobial susceptibility testing

Antimicrobial susceptibility tests are generally performed on all organisms of clinical significance except for a few that have predictable antimicrobial susceptibility patterns (group A streptococci remain universally susceptible to penicillin).

Agar disk diffusion method (Bauer-Kirby method)

- a standardized inoculum of the organism is seeded onto an agar plate.
- antibiotic-impregnated filter paper disks are then placed on the agar surface.
- after 18-24 hr of incubation, the zone of inhibition of bacterial growth around each disk is measured and compared with nationally determined standards for susceptibility or resistance.



Dilution testing

a standard concentration of a microorganism is inoculated into serially diluted concentrations of antibiotic

- the **minimum inhibitory concentration (MIC)** in µg/mL, the lowest concentration of antibiotic required to inhibit growth of the microorganism, is determined.
- the **minimum bactericidal concentration (MBC),** the lowest concentration of antibiotic required to kill the organism.

E-test

is used to measure the MIC of individual antibiotics on an agar plate.

it uses a paper strip impregnated with a known continuous concentration gradient of antibiotic that diffuses across the agar surface, inhibiting microbial growth in an elliptic zone.

the MIC is read off the printed strip at the point at which the zone intersects the strip.

advantages : reliable interpretation, reproducibility, and applicability to organisms that require special

media or growth conditions, including anaerobic bacteria.

Office bacteriology

rapid antigen testing for detection of group A streptococcal pharyngitis.
Susceptibility depends on the type of kit used and on the concentration of streptococci present in the sample. As many as 30% of test results may be false-negative; therefore, all negative results should be confirmed by culture.

Immunological tests

Immunologic tests use an

- antigen to detect antibodies to a pathogen or
- use an antibody to detect an antigen of the pathogen in the patient's specimen.

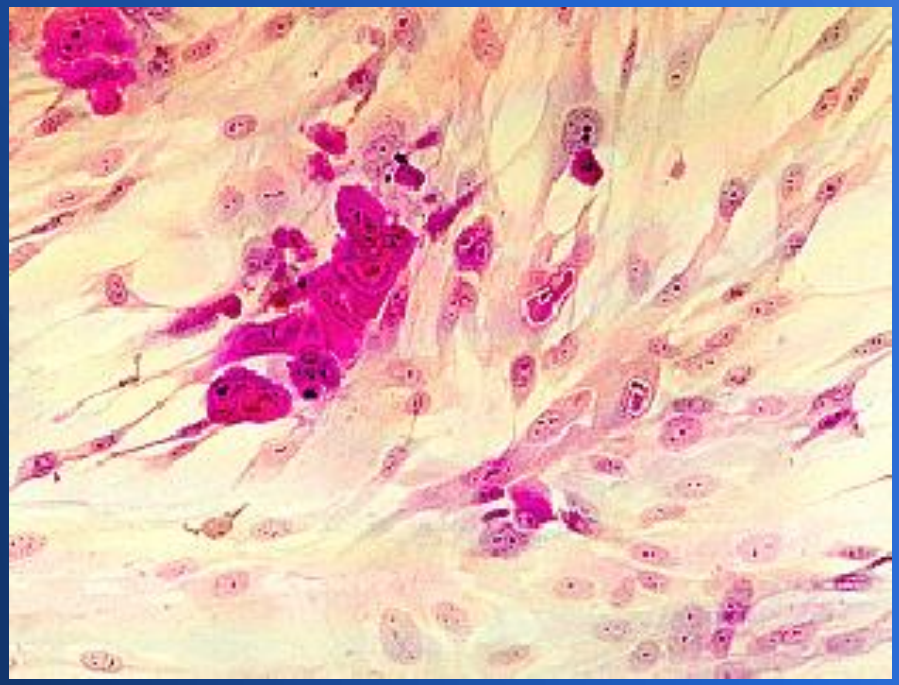
Agglutination tests: In agglutination tests (eg, latex agglutination, coaggregation), a particle (latex bead or bacterium) is coupled to a reagent antigen or antibody. The resulting particle complex is mixed with the specimen (eg, CSF, serum); if the target antibody or antigen is present in the specimen, it cross-links the particles, producing measurable agglutination.

If results are positive, the body fluid is serially diluted and tested. Agglutination with more dilute solutions indicates higher concentrations of the target antigen or antibody. The titer is correctly reported as the reciprocal of the most dilute solution yielding agglutination; eg, 32 indicates that agglutination occurred in a solution diluted to 1/32 of the starting concentration.

Diagnosis of Viral Infection

Laboratory diagnosis of viral infections may be by

- Molecular methods
  - Polymerase Chain Reaction
  - Sequencing (e.g. for sensitivities)
- Electron microscopy
- Antigen detection
- Antibody detection
- Virus culture
  - Detect cytopathic effect or antigen



Viral Serology

Enzyme-Linked Immunosorbent Assays (ELISAs)

- Enzyme reacts with substrate to produce colored product

These tests use antibodies linked to enzymes to detect antigens and to detect and quantify antibodies. The enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are examples. Because sensitivities of most enzyme immunoassays are high, they are usually used for screening. Titers can be determined by serially diluting the specimen as for agglutination tests.

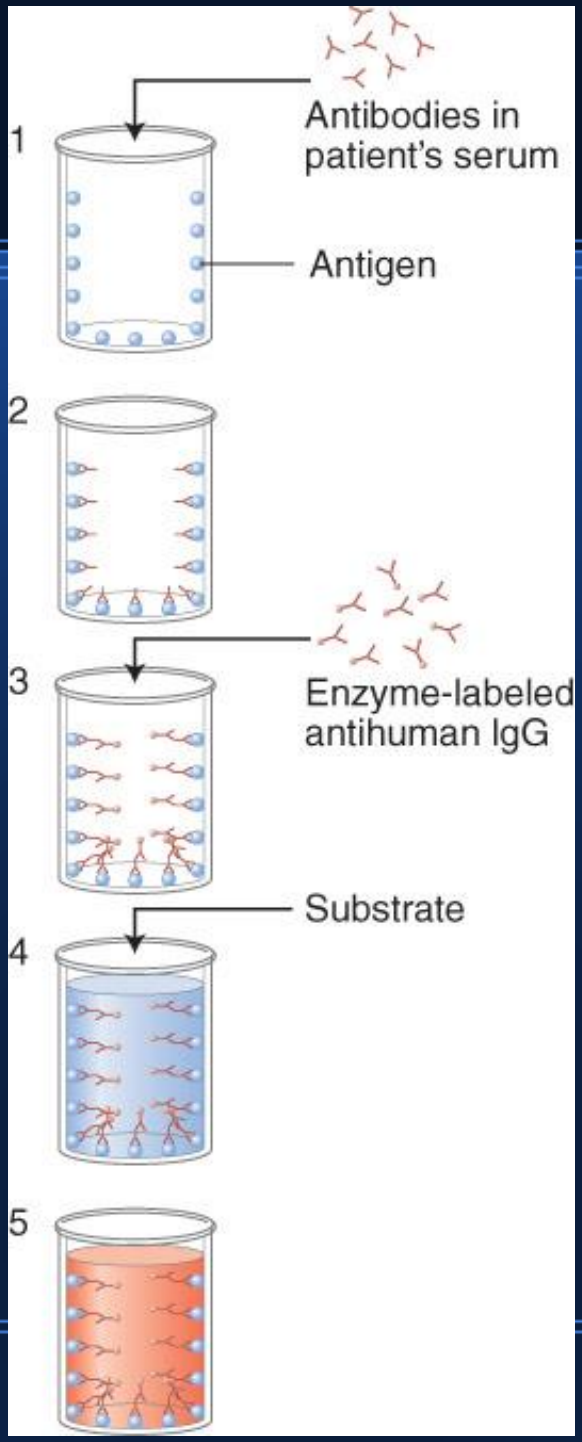
- Very sensitive
  - HIV test
    - If positive twice, Western Blotting is performed next

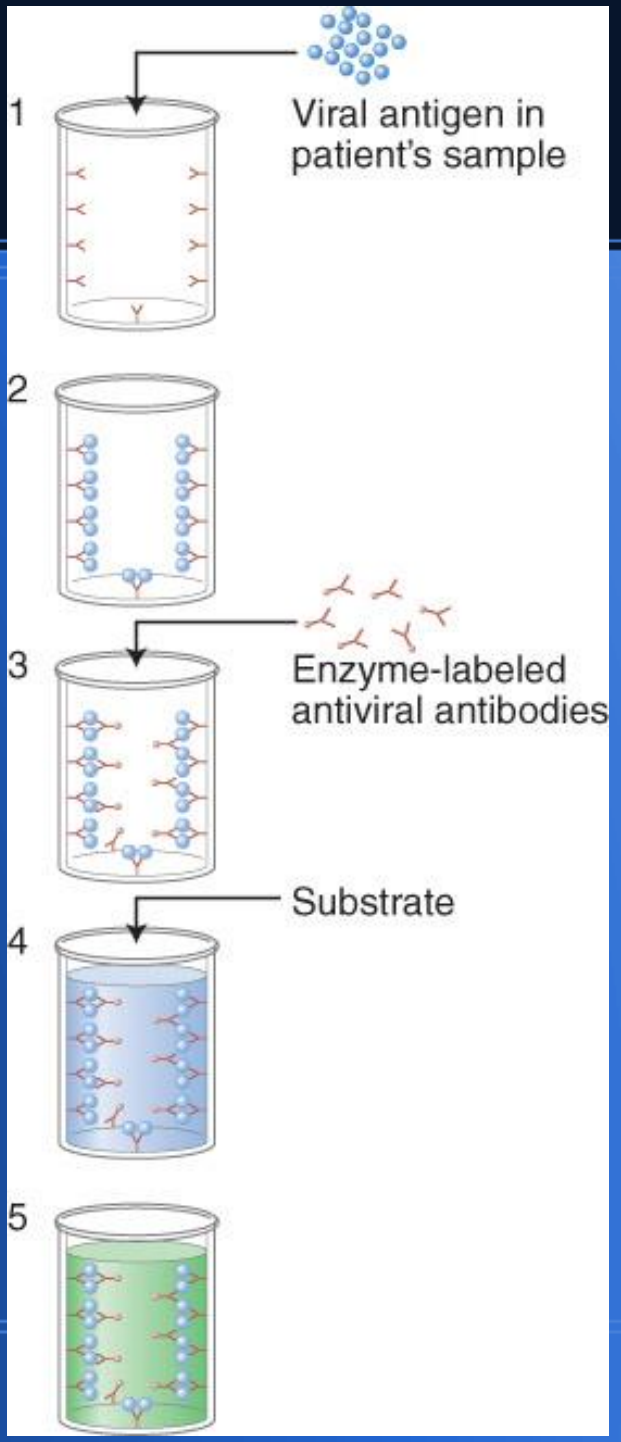
Western blot test: This test detects antimicrobial antibodies in the patient's sample (eg, serum, other body fluid) by their reaction with target antigens (eg, viral components) that have been immobilized onto a membrane by blotting.

it is usually used to confirm a positive result obtained with a screening test.

the recombinant immunoblot assay (RIBA), which use synthetic or recombinant-produced antigens; and immunochromatographic assays, which can rapidly screen specimens for specific microbial antigens or patient antibodies.

ELISA Procedures





Rapid Antigen Tests

Immunofluorescent-antibody (IFA) techniques ; EIA (enzyme immunoassay) use **antibodies** to detect viral antigens directly ,within 2-3 hr after the specimen is received

- respiratory syncytial virus (RSV),
- adenovirus,
- influenza virus
- parainfluenza virus
- varicella-zoster virus (VZV) -IFA
- herpes simplex virus (HSV) -IFA
- cytomegalovirus (CMV)
- rotavirus, - EIA
- noroviruses - EIA
- hepatitis B virus - EIA

Isolation and Identification of viruses

Viruses require living cells for propagation; the cells used most often are human- or animal-derived tissue culture monolayers, such as human embryonic lung fibro-blasts or monkey kidney cells.

In vivo methods for isolation are sometimes necessary (suckling mice inoculation for culture of arboviruses and rabies virus).

Viral growth in susceptible cell cultures can be detected in several ways. Many viruses produce a characteristic **cytopathic effect (CPE)** that is visible by light microscopy under low magnification. For example, RSV and HSV produce multinucleated giant cells and syncytia formation. Other viruses (e.g., influenza and mumps) can be detected by hemadsorption because hemagglutinins on infected cell membranes permit adherence of erythrocytes to infected cells. The most reliable confirmatory method for viral detection in cell culture involves fluorescein- or enzyme-labeled monoclonal antibody staining of infected cell monolayers.

An important technical improvement in respiratory viral cultures is the development of an engineered tissue monolayer (R-mix) for rapid shell vial detection of influenza A and B, respiratory syncytial virus, parainfluenza 1-3, and adenoviruses. Respiratory shell vial cultures have turn-around times of 2 days compared with 2-3 weeks for conventional cultures and are most useful for rapid diagnosis of influenza infections where sensitivity of IFA is low, and rapid detection can result in early institution of appropriate antiviral therapy.

Laboratory diagnosis of parasitic infections

Microscopic examination of clinical specimens:

- *Plasmodium* and *Babesia* can be detected in stained blood smears,
- *Leishmania* in bone marrow smears,
- helminth eggs, *Entamoeba histolytica, Giardia lamblia* cysts, and trophozoites in fecal smears

Serologic tests :

- *Trichinella* and *Toxoplasma*
- intestinal strongyloidiasis.
- because *Giardia* and many worm eggs are shed intermittently into feces, a minimum of three specimens are required for an adequate examination. It is recommended that the three specimens be collected on separate days, preferably on alternate days.

Cryptosporidium and *Cyclospora* are detected by modified acid-fast stain and microsporidia by a modification of the trichrome stain. Detection of certain parasites, especially *Giardia* and *Cryptosporidium,* can be simplified by using sensitive EIA antigen detection tests. Rapid antigen detection ("dipstick") tests for *Plasmodium falciparum* and *P. vivax* are also available with sensitivities and specificities comparable to expert microscopy.

Serologic diagnosis

Serologic tests are primarily used in the diagnosis of infectious agents that are difficult to culture in vitro or detect by direct examination, such as

- Bartonella,
- Legionella,
- *Borrelia* (Lyme),
- Treponema pallidum,
- *Mycoplasma,*
- Rickettsia,
- *Ehrlichia,*
- some viruses (HIV, EBV, hepatitis A and B viruses)
- parasites *(Toxoplasma, Trichinella).*

Serologic diagnosis

Several rapid enzyme-linked immunospot (ELISPOT) assays that detect interferon production by tuberculosis-specific lymphocytes in the patients' blood are undergoing clinical evaluation and may have useful applicability for diagnosis of tuberculosis in children.

Antibody tests may be specific for immunoglobulin G (IgG) or M (IgM) or may measure antibody response regardless of immunoglobulin class. The IgM response occurs earlier in the illness, generally peaking at 7-10 days after infection, and usually disappears within a few weeks but for some infections (e.g., hepatitis A) may persist for months. The IgG response peaks at 4-6 wk and usually persists for life

IgM antibody in most cases = recent infection

a single positive serum specimen is considered diagnostic.

Methods for IgM antibody detection are difficult to standardize, however, and false-positive results frequently occur with some tests.

IgG antibody = new seroconversion or past exposure to the pathogen.

to confirm a new infection - demonstrate either seroconversion or a rising IgG titer.

A fourfold increase in a convalescent titer obtained 2-3 wk after the acute titer is considered diagnostic in most situations.

However, for some infections (e.g., Bartonella, Legionella, and rickettsiae) a single positive IgG titer is sufficient for diagnosis.

Molecular diagnostic techniques

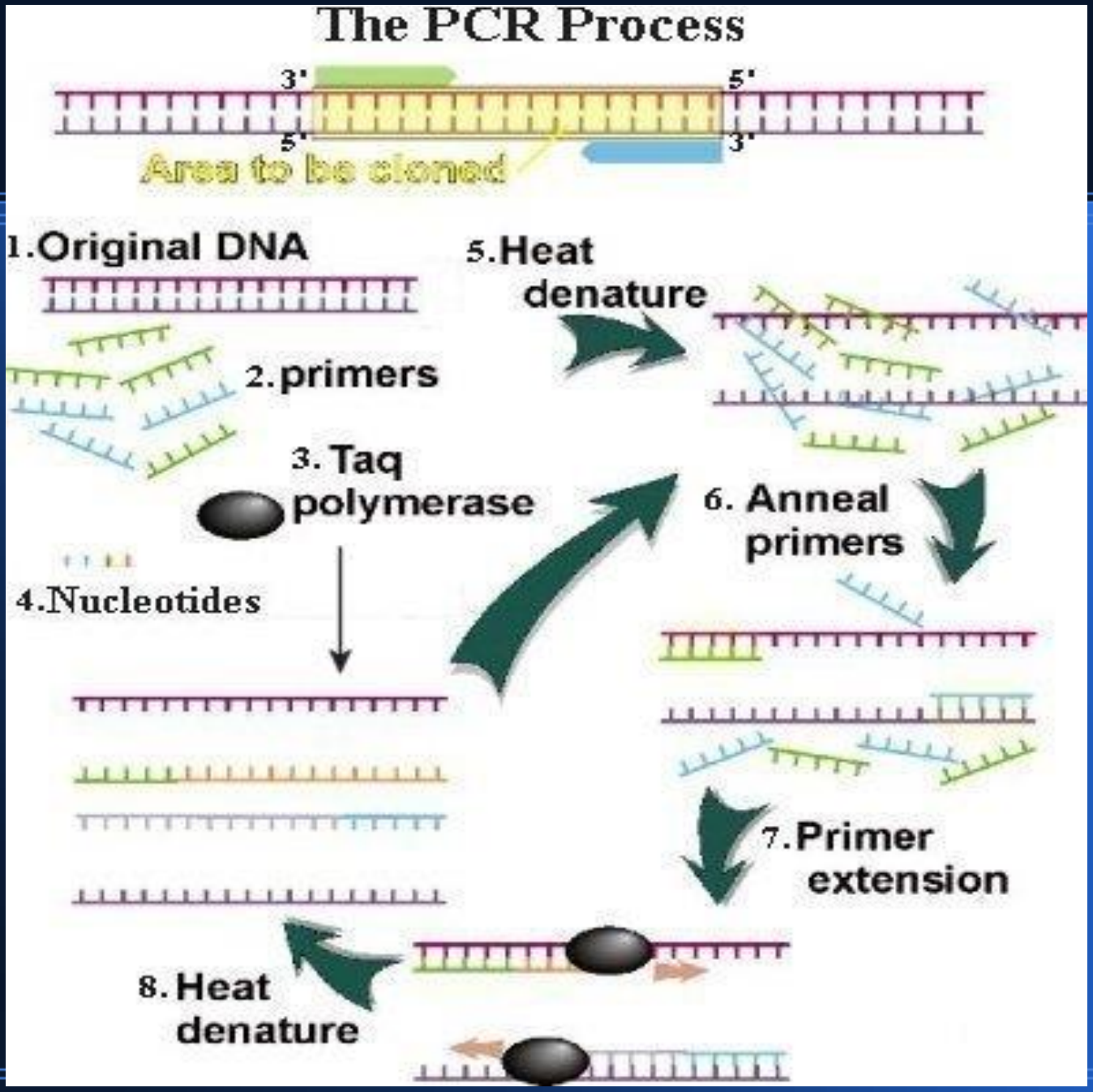
Molecular diagnostic techniques are most useful for detecting and identifying pathogens for which culture and serologic tests are difficult, slow, or not available.

DNA probes for direct detection

Nucleic acid amplification using polymerase chain reaction (PCR).

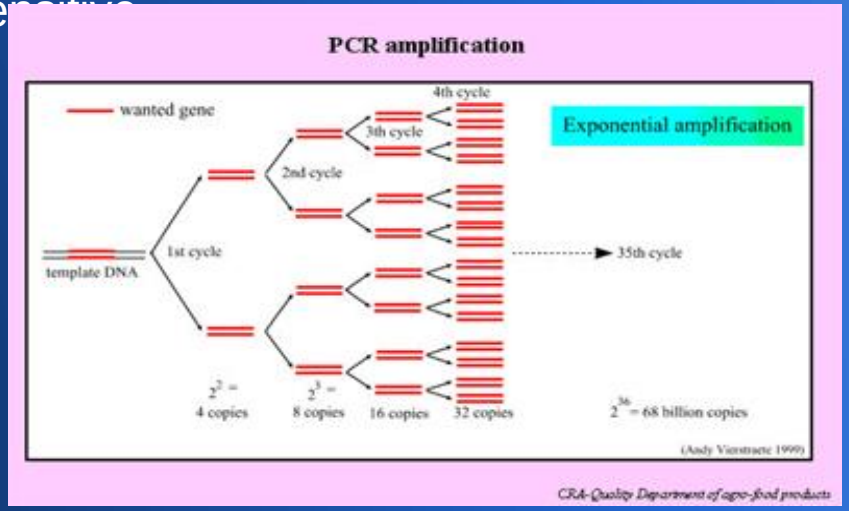
Microbiologic applications of techniques such as miniaturized DNA chip technology and microarrays based on the ability of DNA to find and spontaneously bind to its complementary sequences (mycobacterial DNA in a patient's blood) are also being developed.

DNA probes detect or identify organisms by hybridization of the probe to complementary sequences in DNA or ribosomal RNA. The principal use of DNA probe technology remains rapid identification of organisms that already have been isolated in culture but require additional time-consuming or complex confirmation procedures.


The PCR Process
3'
5'
5'
3'
Area to be cloned
1.Original DNA
2.primers
3. Taq polymerase
4.Nucleotides
5.Heat denature
6. Anneal primers
7.Primer extension
8.Heat denature

Molecular diagnostic techniques

PCR amplification make this the method of choice for **direct** detection of microbial nucleic acid from clinical specimens. The PCR method is based on the ability of thermostable DNA or RNA polymerase to copy targeted gene sequences using complementary nucleotides as primers to amplify a conserved region of the genome. The reaction takes place in a thermal cycler. Each cycle of the reaction theoretically doubles the amount of target nucleic acid, resulting in more than a million-fold amplification after 30 cycles of PCR. The greatest impact of PCR is in clinical virology and mycobacteriology where conventional methods are slow and insensitive.



Pathogens that can be detected by PCR is

- HIV
- hepatitis B and C viruses
- CMV, *M. tuberculosis*
- *C. trachomatis.*
- Experimental PCR protocols for detection of *Bartonella, B. pertussis, Legionella, M. pneumoniae, Chlamydia pneumoniae,* and HSV are available in some reference laboratories.

Laboratory Diagnosis of fungal Diseases

Fungal Specimen Examination :

Fluids should be centrifuged and the deposit examined.

Smears and staining of clinical specimen :

Microscopic Examination:

- KOH mount: allows rapid observation of fungal elements skin, nails and biopsy material
- Calcofluor white mount: for rapid screening of fungal elements from clinical specimen.
- Indian Ink preparation: rapid detection encapsulated yeast.
- Gram Stain: most of the fungi are gram-positive.
- Periodic Acid Schiff Stain (PAS).
- Hematoxylin and eosin Stain (H&E).
- Methenamine Silver Stain (GMS).

Specimen Processing :

- CULTURE :
  - A. Culture Media :
    1. Sabouraud s dextrose agar (SDA).
    2. SDA with antibiotics & actidione
    3. Czapek dox agar.
    4. Corn meal agar.
    5. Brain Heart infusion agar.

Biochemical test :

- Sugar Assimilation and Sugar Fermentation test .
- Urease test.

Serological test : Antigen or Antibody detection.

Complement fixation: - measures complement-consuming (complement-fixing) antibody in serum or CSF. The test is used for diagnosis of some viral and fungal infections, particularly coccidioidomycosis. The specimen is incubated with known quantities of complement and the antigen that is the target of the antibody being measured. The degree of complement fixation indicates the relative quantity of the antibody in the specimen. The test can measure IgM and IgG antibody titers or can be modified to detect certain antigens.

Molecular techniques like PCR.


Thank you