AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 7.

Microbiology diagnosis of diseases, caused by *Corynebacteria*, *Bordetella*, *Haemophilus*, *Gardnerella* and *Legionella* genus

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. Bacteria from the genus *Corynebacterium*. Morpho-biological characteristics of the causative agent of diphtheria, distinguishing features from diphtheroids, pathogenicity factors, mechanism of action of *C.diphtheriae* toxin, pathogenesis of diphtheria.

• Microbiological diagnostic methods of diphtheria

• Principles of specific treatment and prevention of diphtheria

• Diphtheroids and their role in human pathology

2. *Bordetellas*, classification, morpho-biological characteristics. Morpho-biological features, distinguishing features, pathogenicity factors, pathogenesis of the causative agents of pertussis and pertussis-like diseases.

• Methods of microbiological diagnosis of pertussis and pertussis-like disease

• Principles of specific treatment and prevention of whooping cough

3. Hemophilic bacteria. *Haemophilus influenzae*, morpho-biological characteristics, pathogenicity factors. Pathogenesis, microbiological diagnosis of diseases caused by it

• *H.ducreyi*, morpho-biological characteristics and microbiological diagnosis

4. *Gardnerella vaginalis*, morpho-biological characteristics, pathogenetic characteristics, microbiological diagnosis

5. *Legionella*, morpho-biological characteristics, pathogenicity factors. Legionellosis pathogenesis, clinical forms, microbiological diagnosis

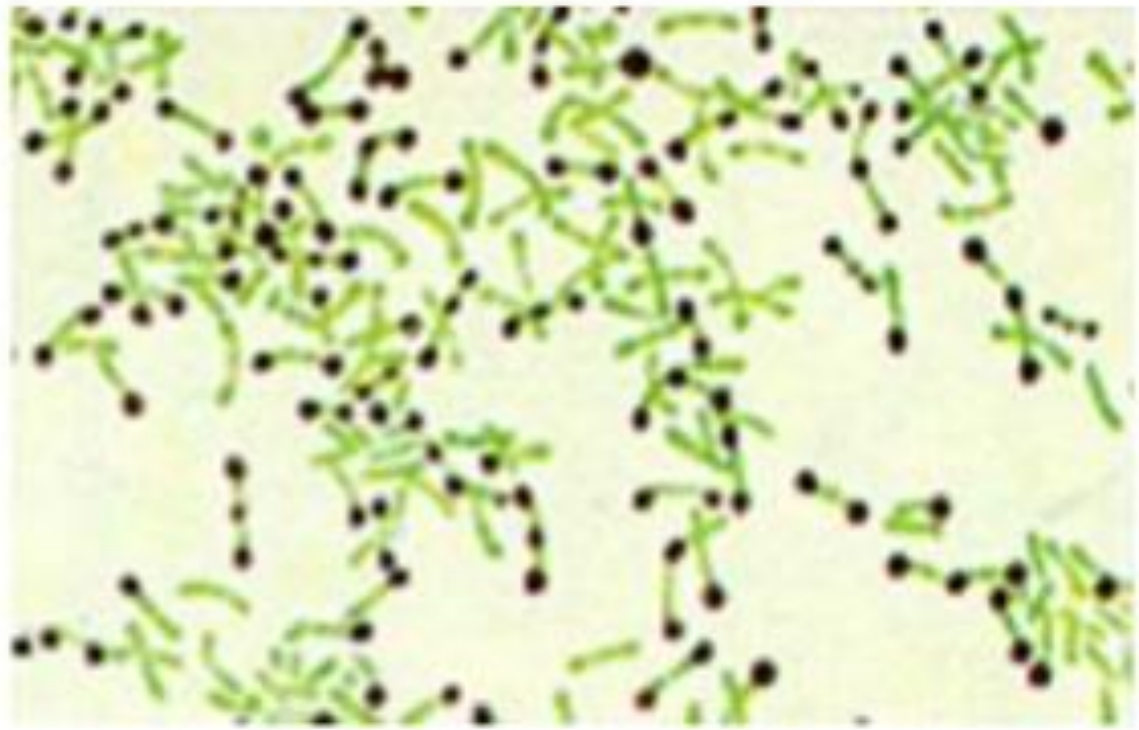
Purpose of the lesson:

- To acquaint students with the morpho-biological characteristics of corynebacteria, bordetella, hemophilic bacteria, gardnerella and legionella, pathogenesis of diseases caused by them, microbiological diagnosis, specific treatment and prevention principles.

Corynebacterium - Taxonomy

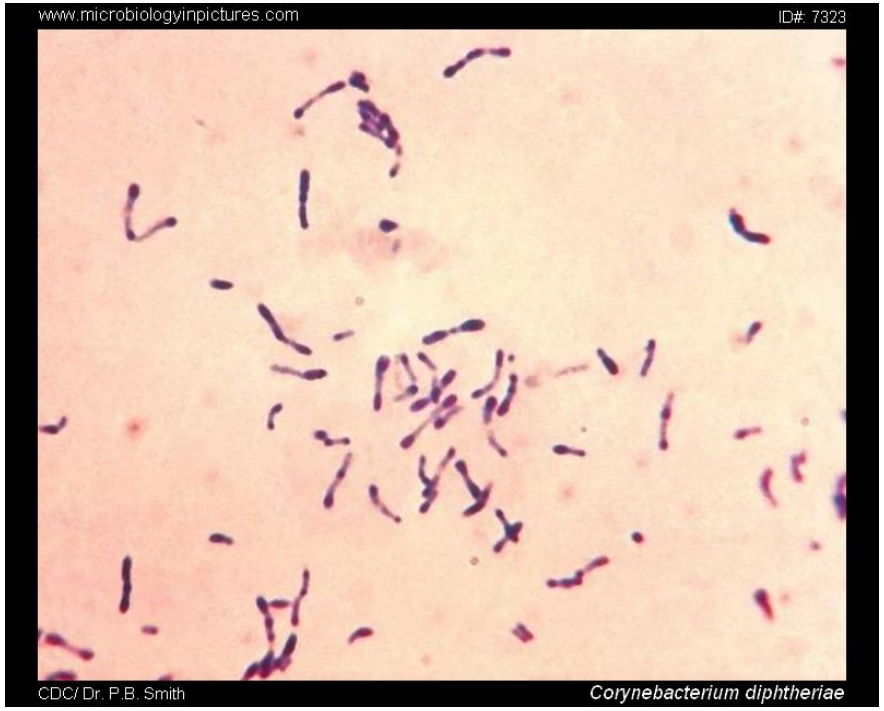
- (Domain): Bacteria
- (Kingdom): Actinomycetota
- (Class): Actinomycetia
- (Order): Mycobacteriales
- (Family): Corynebacteriaceae
- (Genus): Corynebacterium
- : (Species): ***C. diphtheriae***

CORYNEBACTERIUM DIPHTHERIAE



MORPHOLOGY

- Slender Gram-positive rods, pleomorphic; easily decolousised;
- 0.6-0.8µ diameter and 3-6 µ length;
- Irregular swelling at one or both ends ('club shaped');
- Non-capsulate, Non-sporing and nonmotile
- Granules containing polymetaphosphate are seen in the cells;
- Take up bluish purple color against lightly stained cytoplasm, when stained with Loeffler's Methylene Blue, and hence called 'Metachromatic granules';
- Also called, 'volutin granules' or 'Babes Ernst granules';
- They are often situated at poles- 'polar bodies'



MORPHOLOGY

- Special stains for demonstrating the granules :
  - Albert's stain
  - Neisser's stain
  - Ponder's stain
- The bacilli are arranged in pairs, palisades or small groups; the bacilli lie at various angles to each other, resembling the letters, V or L;
- This is called, "Chinese letter pattern" or "cuneiform pattern";

CULTURAL CHARACTERISTICS

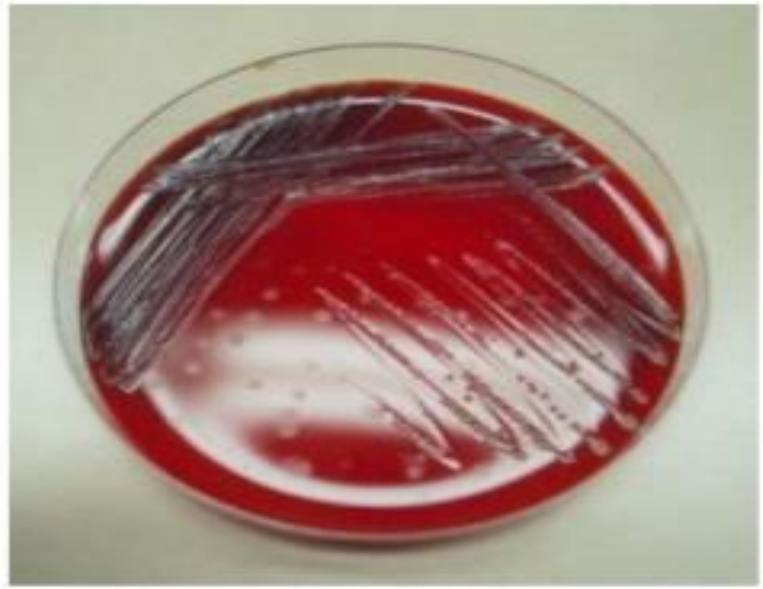
- Aerobe and facultative anaerobe;
- Optimum temperature is 37^0C
- Growth scanty on ordinary media;
- **Enrichment with:** blood, serum or egg is necessary for good growth;
- Potassium tellurite(0.04%) acts as a *'selective agent',* as it inhibits growth of most oral commensals and retards the growth of *Candida albicans* and *S.aureus;*

MEDIA FOR CULTIVATION

- Blood agar
- Loeffler's serum slope
- Tellurite blood agar

COLONY CHARACTERISTICS

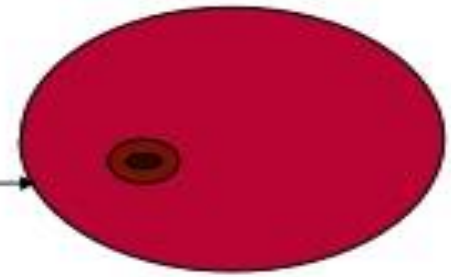
- Blood agar : small, granular and gray with irregular edges; Hemolysis may or may not present;
- Loeffler's serum slope:
  - Very rapid growth;
  - Colonies in 6-8 hrs
  - Initially circular white opaque colonies and acquire yellowish tint on incubation



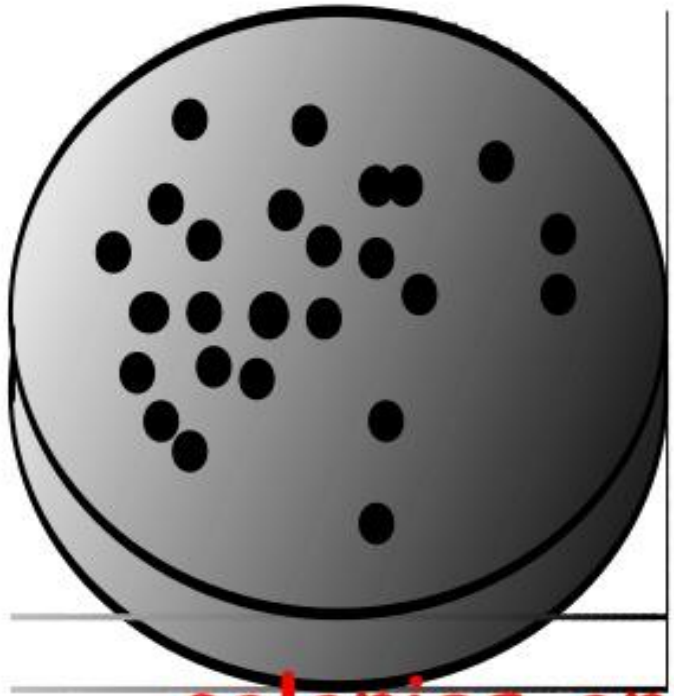
COLONY CHARACTERISTICS

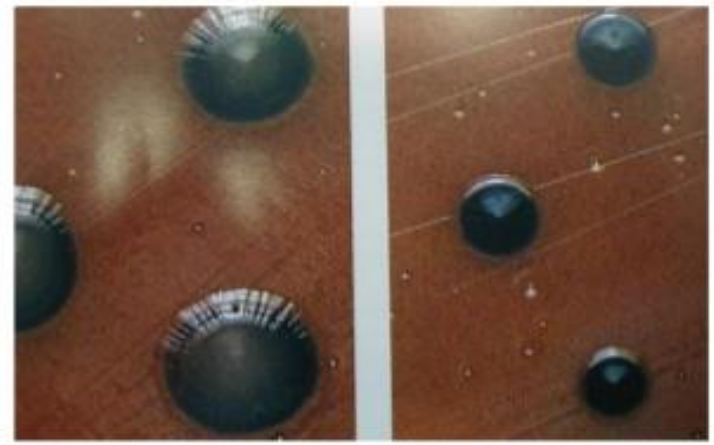
- Tellurite blood agar:
  - Growth slow; colonies seen after 48 hrs;
  - The colonies are brown to black with a brown-black halo because the tellurite is reduced to metallic tellurium;
  - Staphylococcus also produce such colonies

A diagrammatic representation →



Colonial morphology





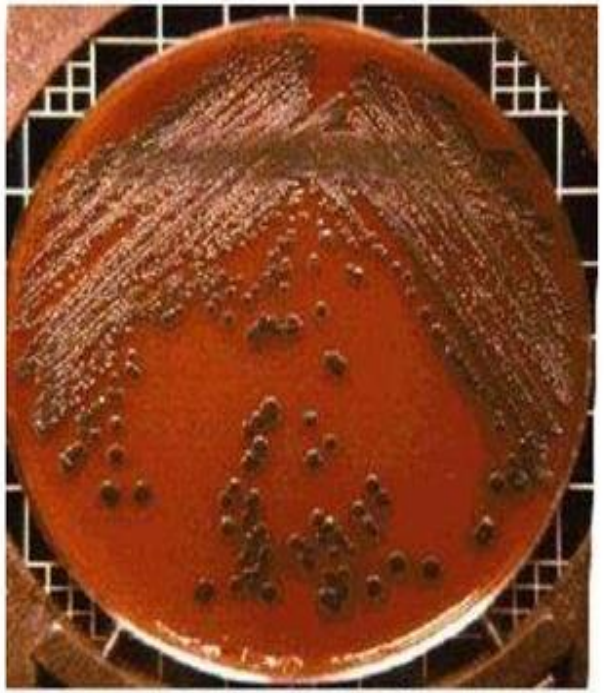
colonies on tellurite agar

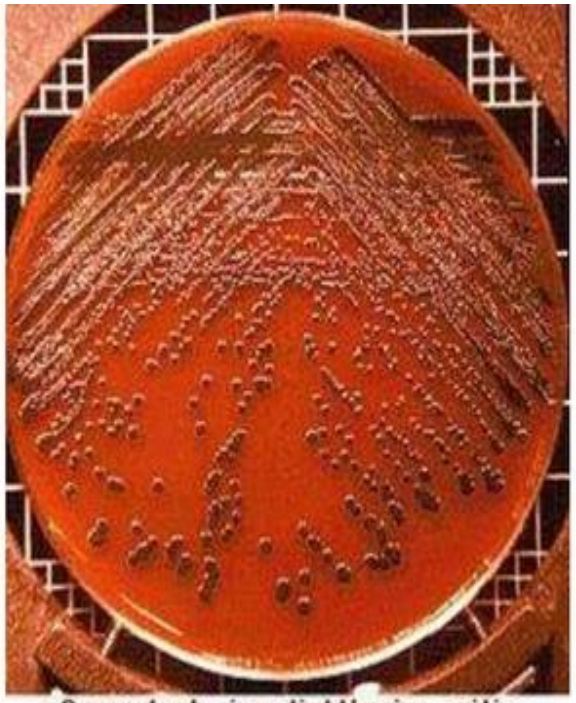
BIOTYPES

- **McLeod and Anderson** classified diphtheria bacilli, based on the colony characteristics on Tellurite medium and other properties like biochemical reactions and severity of disease;
- 3 biotypes :
  - *gravis*
  - *intermedius*
  - *mitis*
- 4th biotype : *belfanti* has also been described

Feature	gravis	intermedius	mitis
Morphology	shot rods, few granules some degree of pleomorphism	long barred forms poor granulation Pleomorphism	long curved prominent granules Pleomorphism
Colony on tellurite blood agar (48 hrs)	**Daisy head colony** (flat colony with raised dark centre and crenated edge; radial striations)	**Frog's egg colony** (dull granular centre with glistening periphery and lighter ring near edge)	**Poached egg colony** (shiny , flat with central elevation)
Consistency of the colonies	Brittle not easily emulsifiable	intermediate	soft, buttery easily emulsifiable
Hemolysis	**Variable**	**nonhemolytic**	**hemolytic**
Glycogen/ starch fermentation	Positive	Negative	Negative

C.diptheriae - biotypes
cultural characteristics


Corynebacterium diphtheriae, gravis
Chocolate tellurite agar


Corynebacterium diphtheriae, mitis
Chocolate tellurite agar


Corynebacterium diphtheriae, intermedius
Chocolate tellurite agar

a) gravis **b) mitis** **c) intermedius**

BIOCHEMICAL REACTIONS

- Hiss serum sugars – for testing fermentation reactions;
- Ferment- *glucose, galactose, maltose and dextrose*; but not *lactose, sucrose, mannitol;*
- Proteolytic activity is absent;
- Do not hydrolyse urea;
- Do not form phosphatase;
- Produce cystinase (halo on Tinsdale's medium)

Corynebacterium spp. - biochemical characteristics

Species	Fermentation				Nitrate reductase
	Cystine	Carbamide	Glucose	Starch	
C.diphtheriae					
gravis	+	-	+	+	+
mitis	+	-	+	-	+
intermedius	+	-	+	-	+
belfanti	+	-	+	-	-
C.pseudodiphthericum	-	+	-	-	+
C.xerosis	-	+	+	-	+
C.ulcerans	-	+	+	-	+
C.jeikeum	-	+	+	-	-
C.sistidis	-	+	+	+	-
C.minitissumum	-	+	+	-	-

RESISTANCE

- Cultures remain viable for 2-3 wks at 25-30^{0}C
- Destroyed by heat
- Resistant to light, desiccation or freezing;
- Easily destroyed by antiseptics
- Susceptible to – Penicillin, erythromycin and broad spectrum antibiotics;

ANTIGENIC STRUCTURE AND TYPING

- Serotyping : Antigenically heterogenous
  - gravis: 13 types
  - intermedius : 4 types
  - mitis : 40 types
- Bacteriophage typing : 15 types
- Bacteriocin typing : diphtheriocin typing

Virulence Factors

1. Diphtheria toxin !!!

- blocks protein synthesis

2. Dermonecrotic toxin

- sphingomyelinase
- increases vascular permeability

3. Hemolysin

4. Cord factor -Toxic trehalose

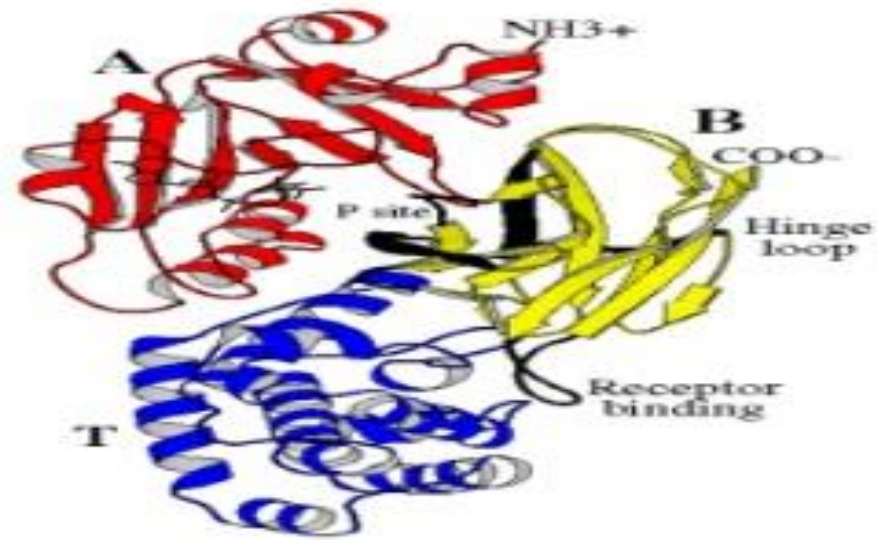
- corynemycolic acid, corynemyolenic acid

VIRULENCE FACTORS

- Virulent strains of diphtheria bacilli produce a very powerful exotoxin.
- The 'virulence' of diphtheria bacilli is due to their capacity to-
  - Establish infection and growing rapidly
  - Quickly elaborate an exotoxin
- Avirulent strains are common among convalescents , contacts and carriers, particularly those with extra-faucial infection

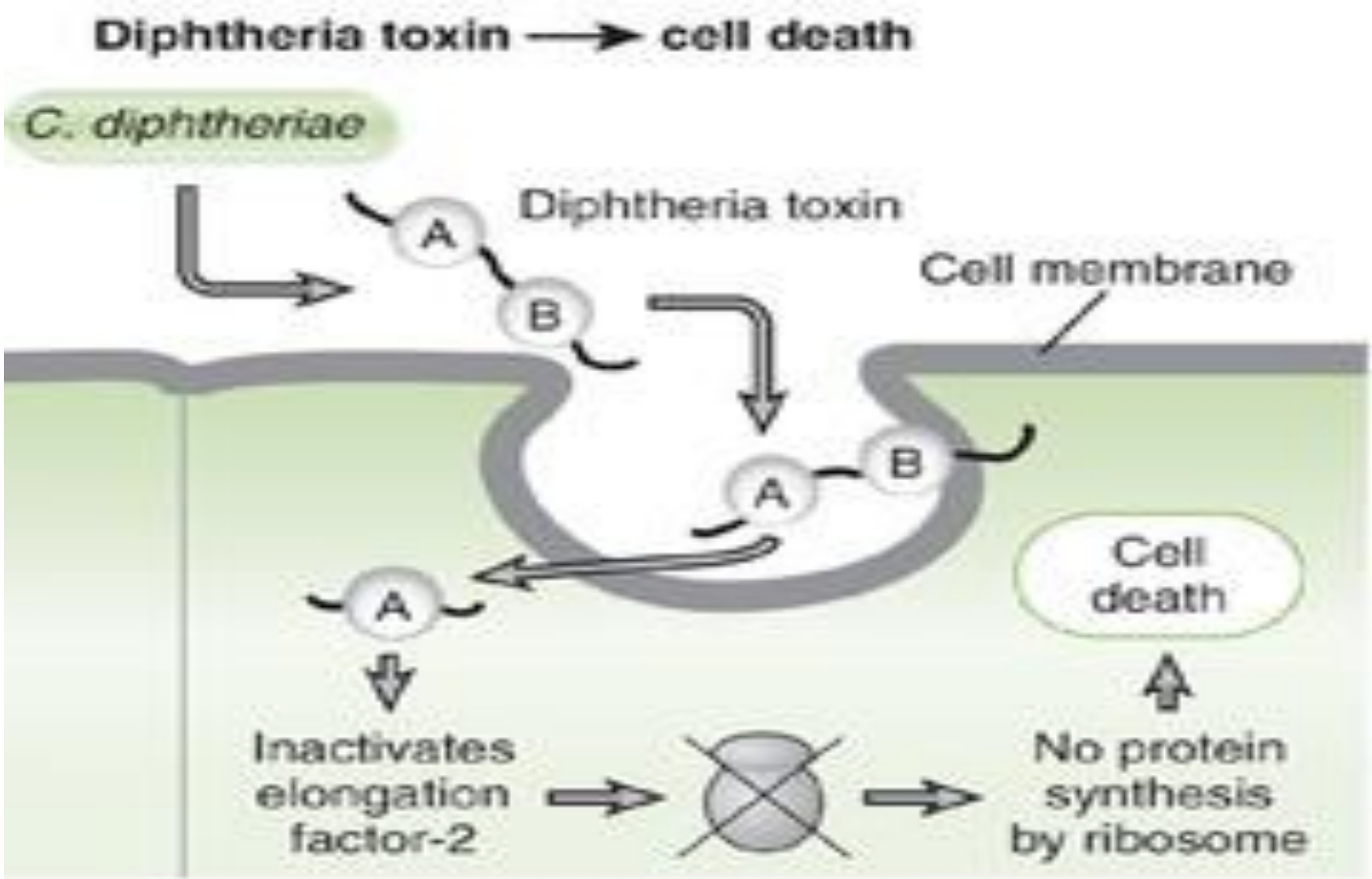
DIPHTHERIA TOXIN

- The pathognomonic effects are due to the toxin;
- Almost all the gravis and intermedius strains and 80-85% of mitis strains are toxigenic
- Toxin is a protein;
- Mol. Wt.: 62,000
- Two fragments, A and B;
- Extremely potent :
  - 0.1 µg lethal to guinea pig
- Inactive when released



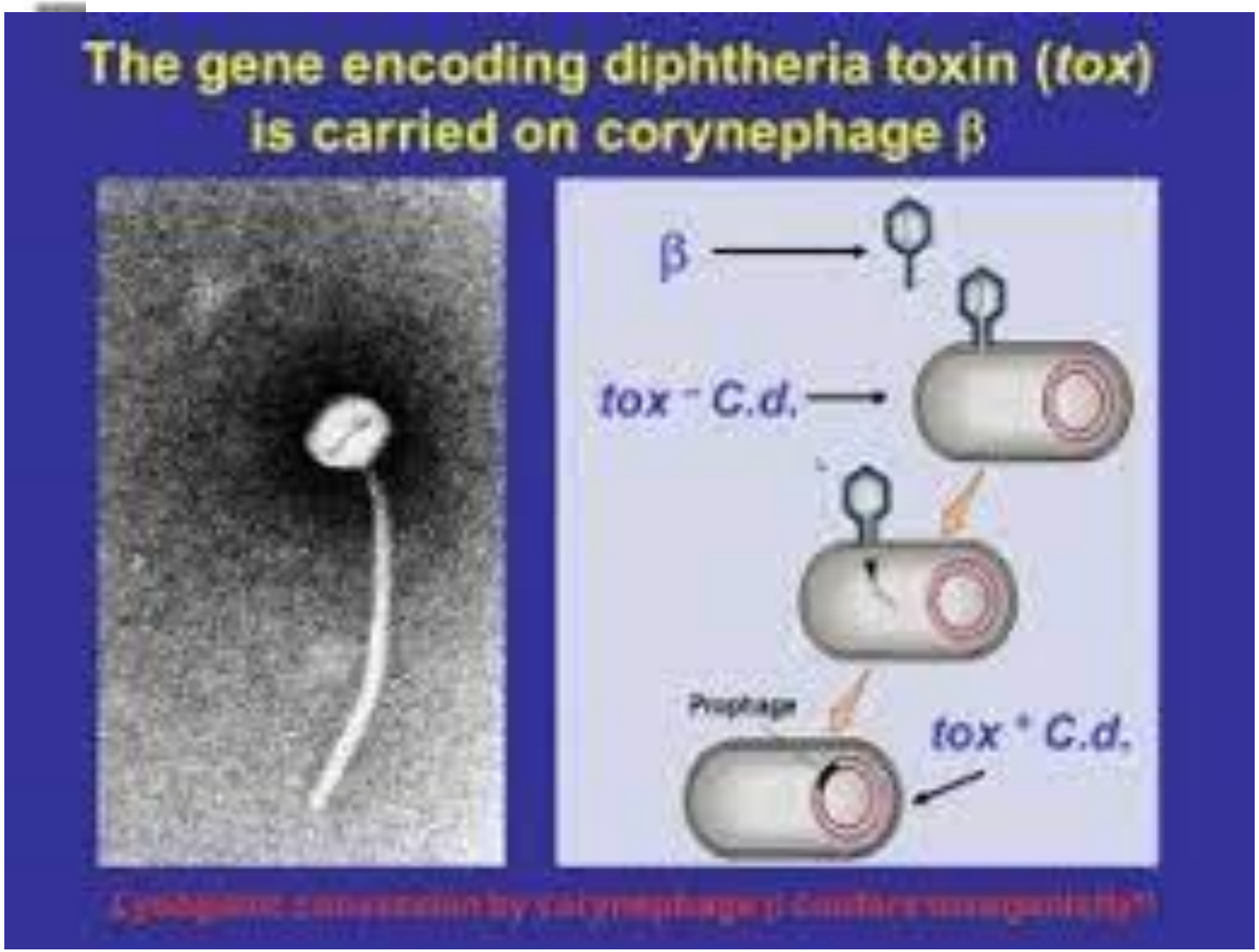
Toxin – mechanism of action

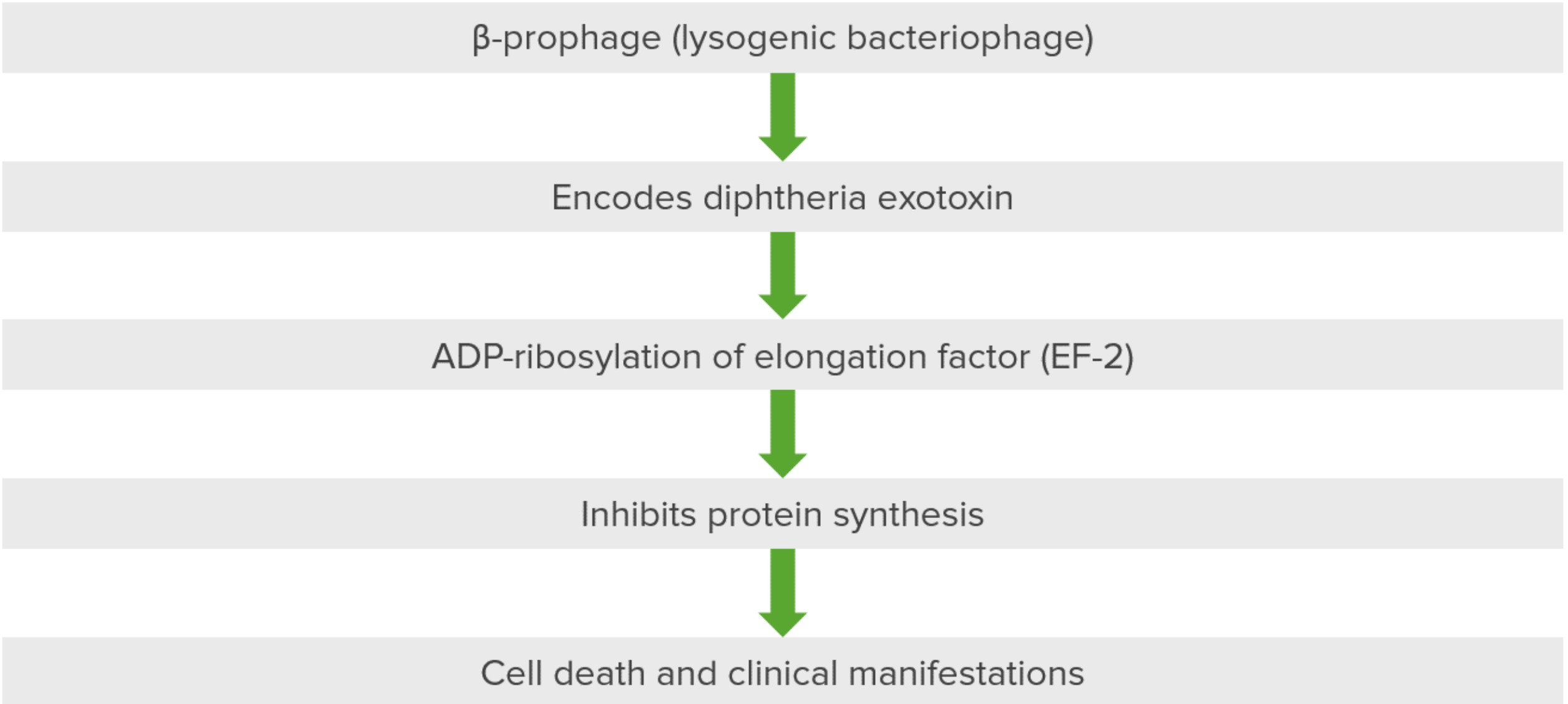
- Fragment B : binds to a cell surface receptor and helps in transport of toxin into the cell;
- After entering the cell, A subunit is released ;
- A subunit catalyses the transfer of 'adenosine diphosphate ribose (ADPR)' from NAD+
- ADPR binds with the elongation factor EF 2
- "ADPR-EF2" complex is inactive → protein synthesis stops abruptly → necrotising and neurotoxic effects of the toxin;


Diphtheria toxin → cell death
C. diphtheriae
Diphtheria toxin
A
B
Cell membrane
B
A
A
Cell death
Inactivates elongation factor-2
No protein synthesis by ribosome

Diphtheria Toxin

- Blocks protein synthesis
- Protein 63Kd
- controlled by Tox gene
- lysogenic phage Beta-corynephage
- Expressed if [iron] low
- 2 components A-B




β-prophage (lysogenic bacteriophage)
Encodes diphtheria exotoxin
ADP-ribosylation of elongation factor (EF-2)
Inhibits protein synthesis
Cell death and clinical manifestations

PATHOGENICITY

- Commonest site of infection: Upper respiratory tract (fauces, larynx,nose)
- Ocassionally, other cutaneous or mucocutaneous areas (otitic/conjunctival/ genitovulval/vaginal/ prepucial/skin)
- Faucial diphtheria is the commonest type;
- Sore throat is frequently the presenting symptom;

PATHOLOGY

- After infection, the bacilli multiply on the mucous membrane or skin abrasion;
- The toxigenic strains start producing toxin;
- Diphtheria is a 'toxemia';
- The bacteria confine to the site of entry but the exotoxin is absorbed into the mucus membrane and causes destruction of epithelium and a superficial inflammatory response;

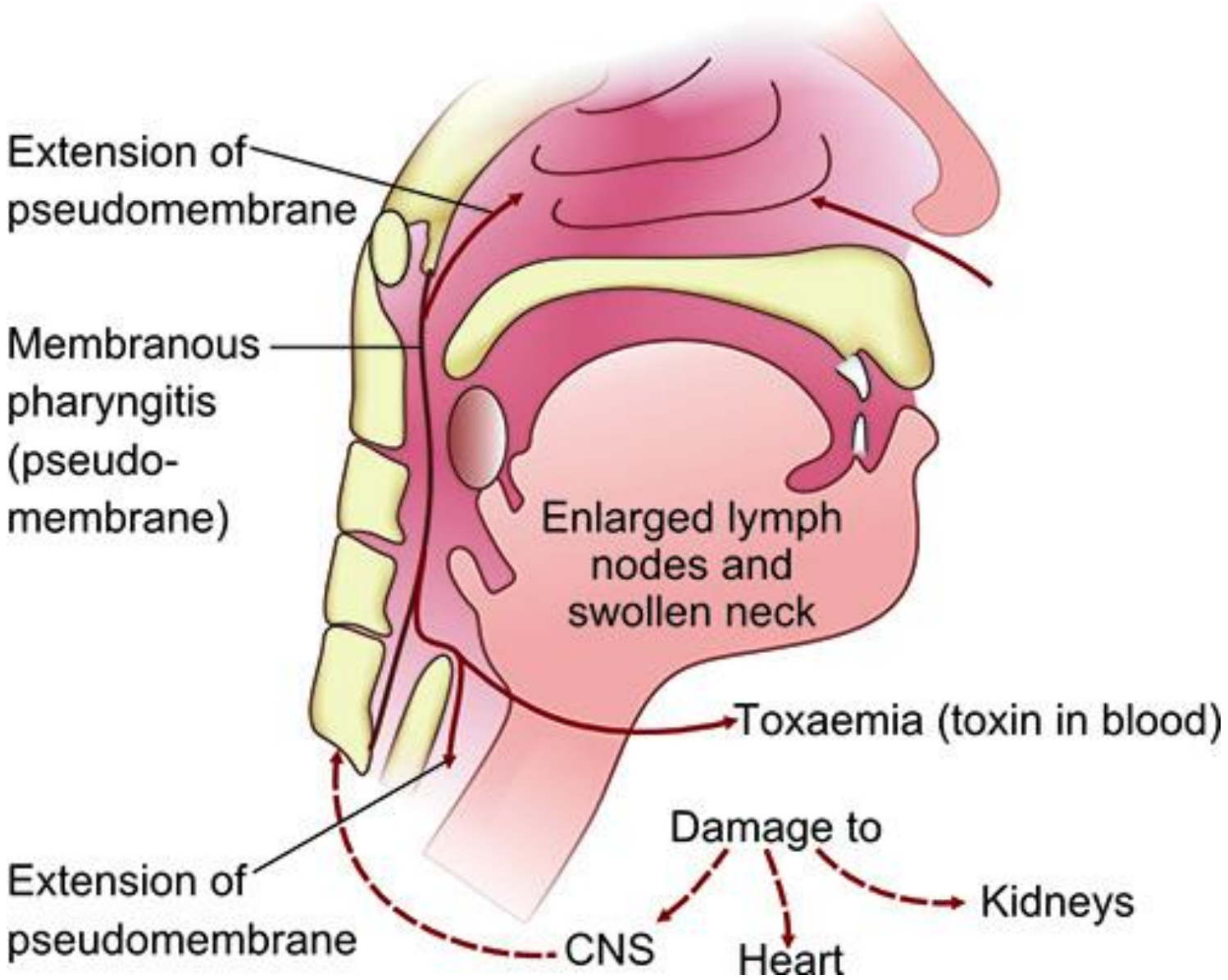
PATHOLOGY

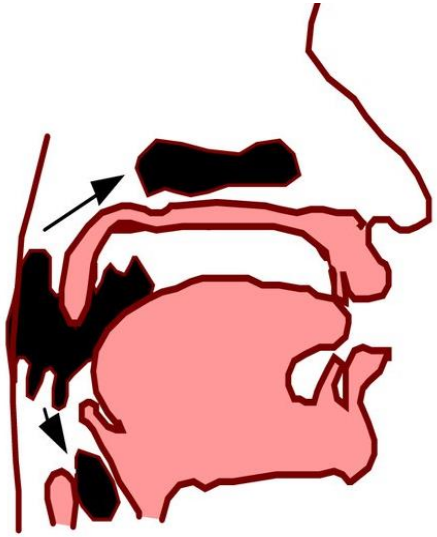
- The toxin causes local necrotic changes;
- The resulting fibrinous exudate, together with the epithelial cells, leucocytes, erythrocytes and bacteria constitute : "pseudomembrane"
- Any effort to remove it will tear off capillaries beneath it and cause bleeding;
- Mechanical complications are due to pseudomembrane and systemic effects are due to the toxin;

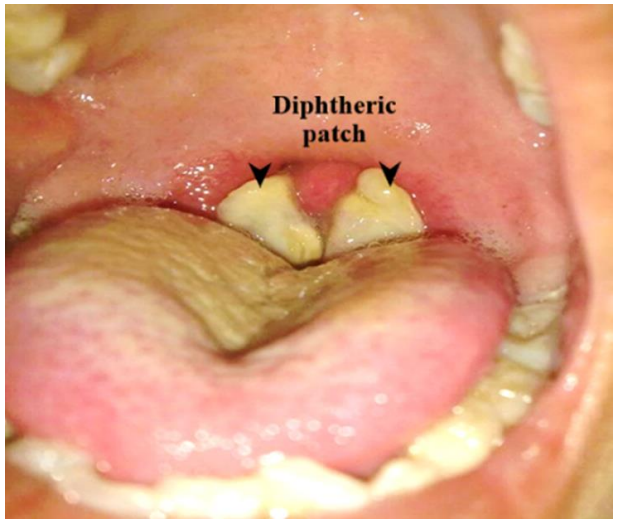
Toxin-systemic absorption

- The bacilli continue to produce the toxin;
- The toxin is absorbed systemically and damages heart muscle, liver, adrenals etc.;
- The toxin also cause nerve damage, especially of soft palate(palatine) and eye muscles (ciliary);
- Toxin absorption is negligible in case of skin infection with toxigenic strains;
- Nontoxigenic strains can also produce local disease but systemic effects are absent;

DİPHTHERİA - PATHOGENESİS





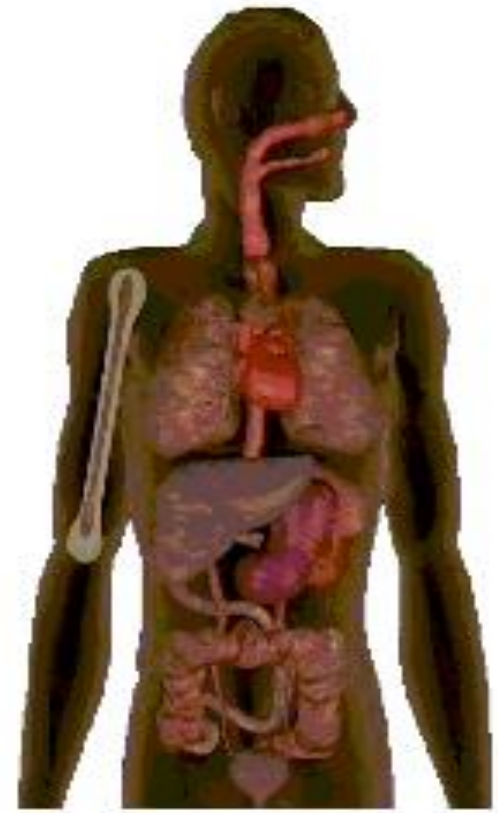


CLINICAL DISEASES

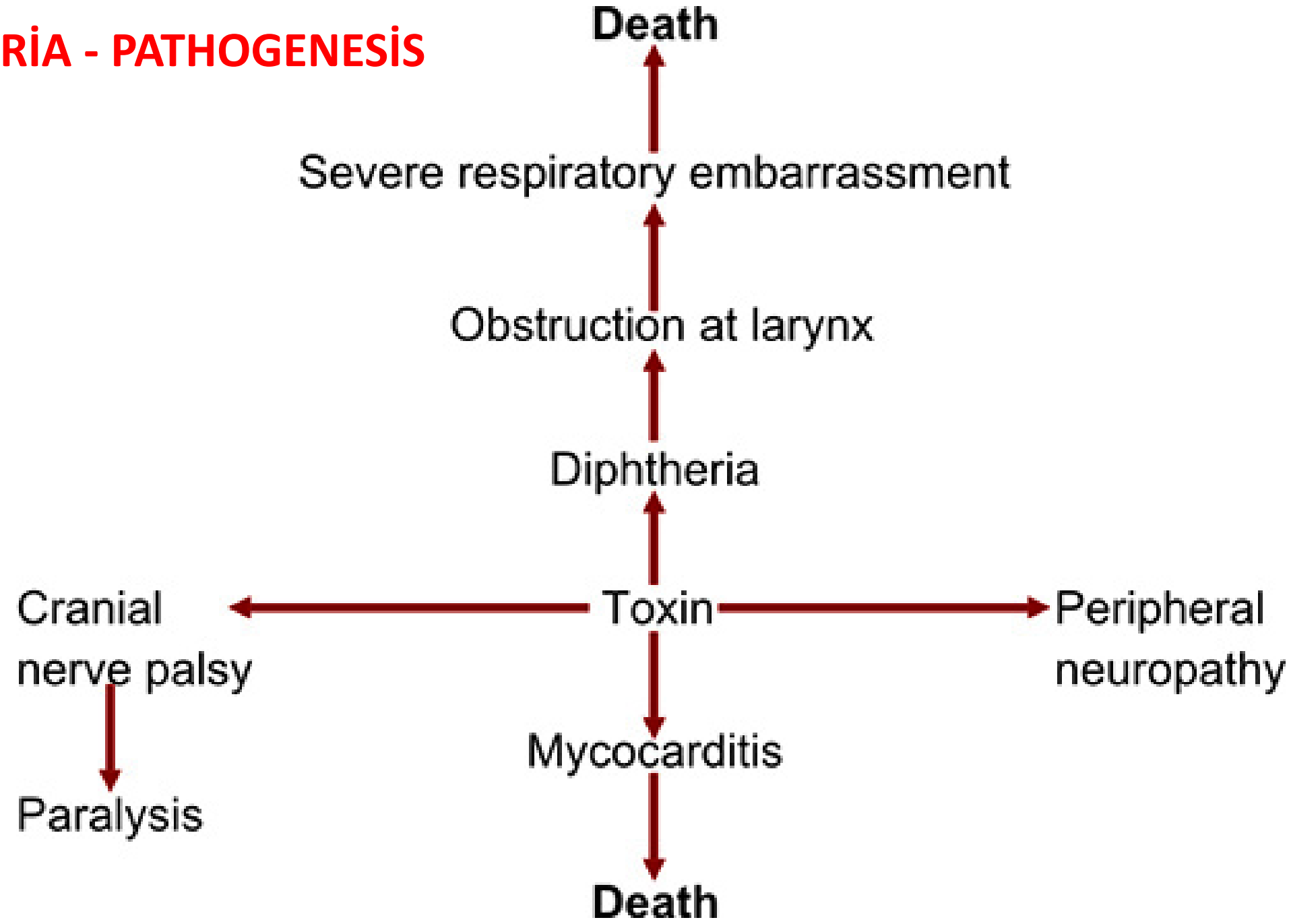
- Incubation period : usually 3-4 days;
- Acute infection : in the form of –
  - **Membranous tonsillitis**
  - **Nasal infection**
  - **Laryngeal infection**
  - **Skin infection –uncommon;**

Diphtheria

- Nasopharyngeal diphtheria
  - Pharyngeal
  - Laryngeal
- Cutaneous diphtheria
- Systemic complications

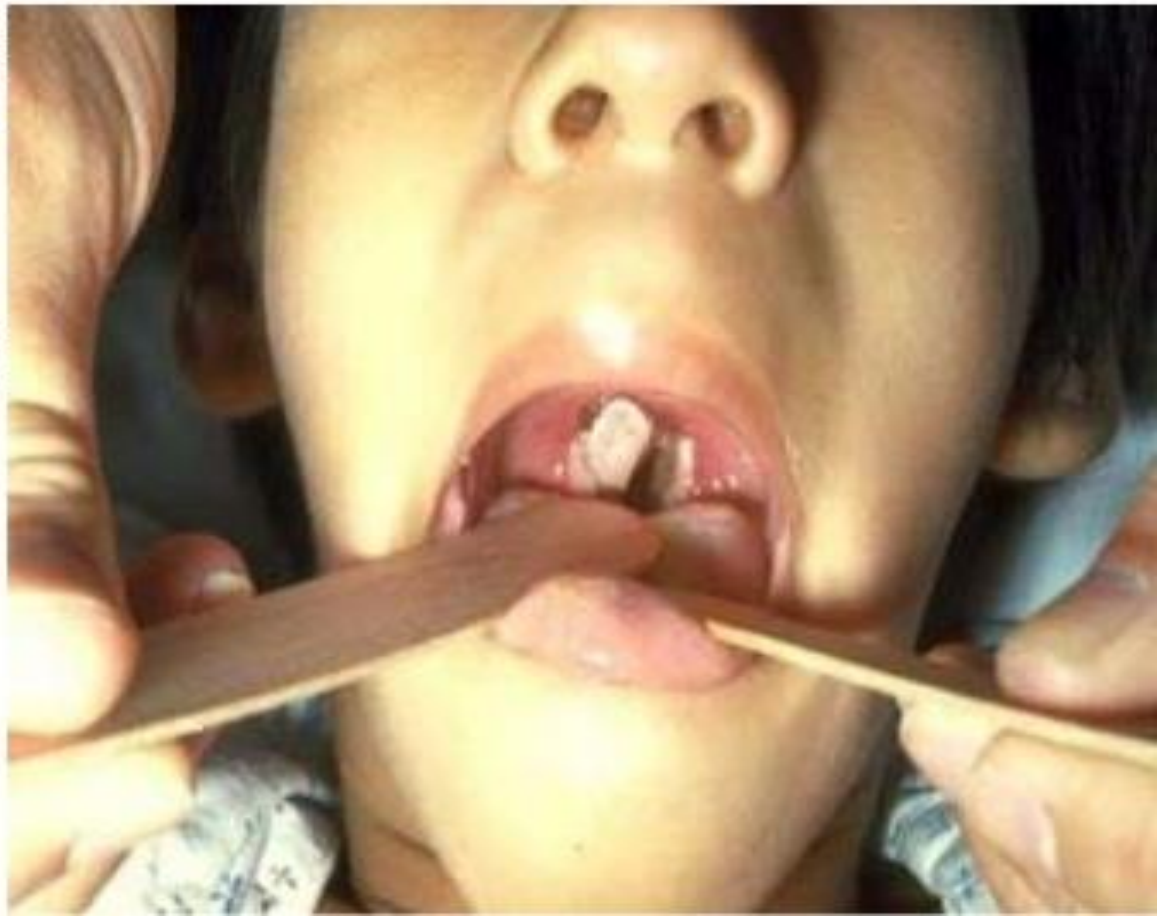


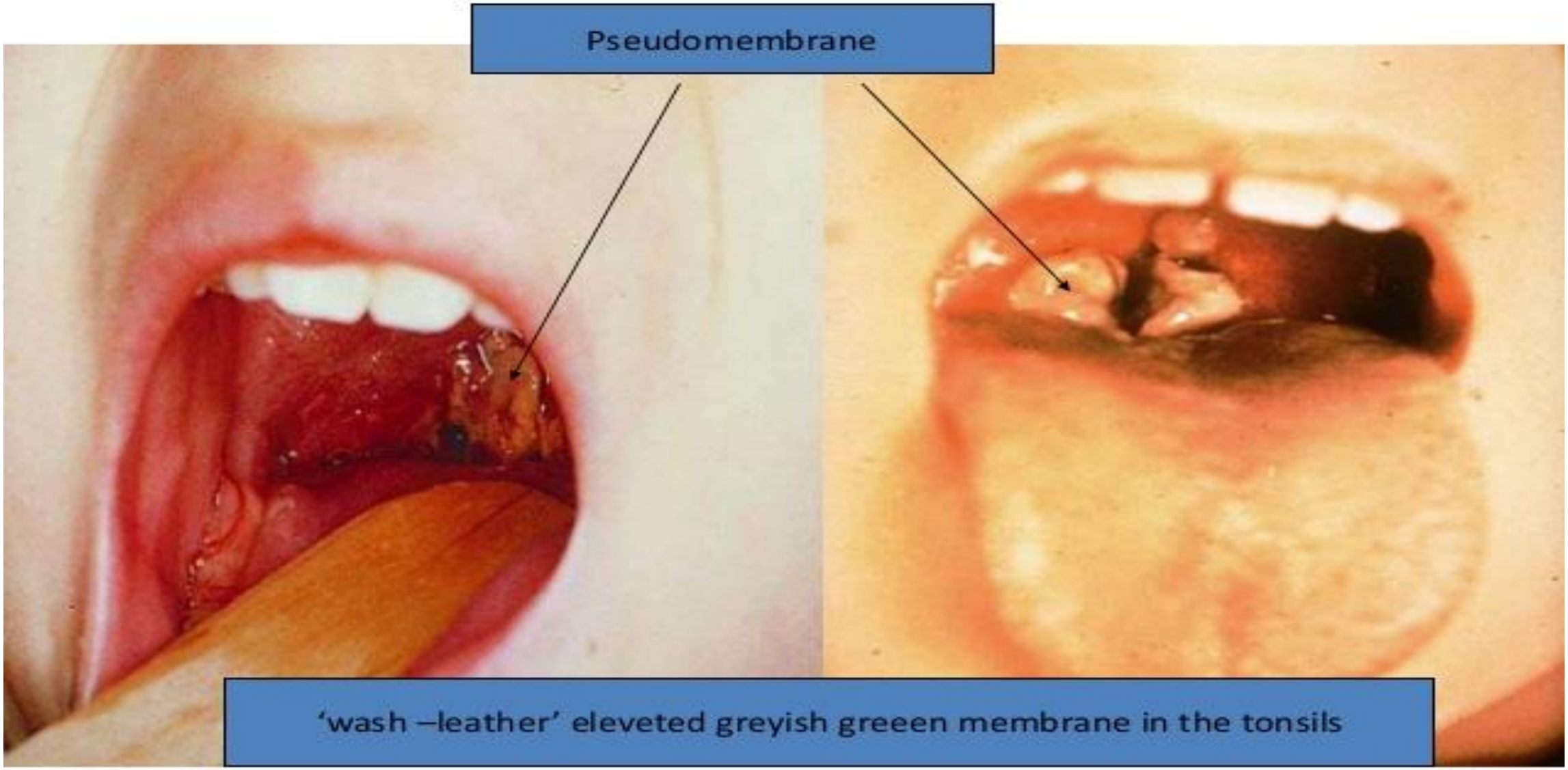
DİPHTHERİA - PATHOGENESİS



CLINICAL DISEASES

- Characteristic feature is : 'wash –leather' eleveted greyish greeen membrane in the tonsils with a well defined edge surrounded by a zone of inflammation;

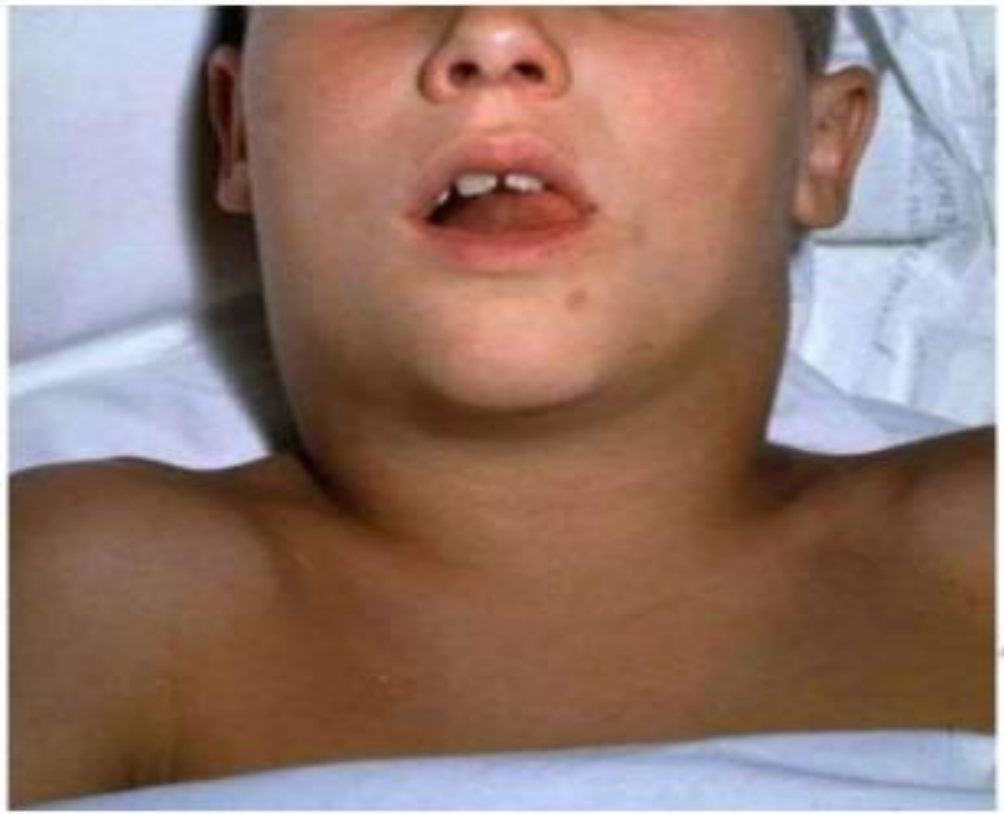


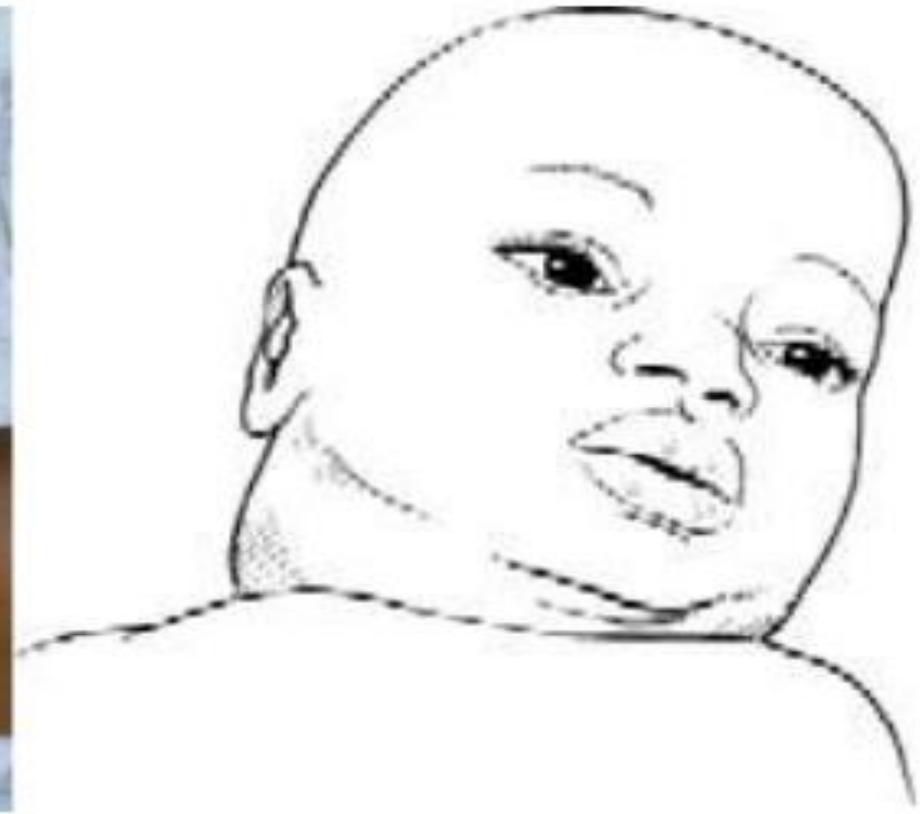

Pseudomembrane
'wash –leather' eleveted greyish greeen membrane in the tonsils

CLASSIFICATION BASED ON CLINICAL SEVERITY

- Malignant or hypertoxic:
  - 'Bull neck' due to marked adenitis in neck;
  - Severe toxemia
  - Circulatory failure
  - Death
  - Paralytic squealae in survivors
- Septic : ulceration, cellulitis and gangrene around pseudomembrane;
- Hemorrhagic: bleeding from the edge of pseudomembrane, epistaxis, purpura etc.

Bull neck : due to cervical adenitis and edema of neck



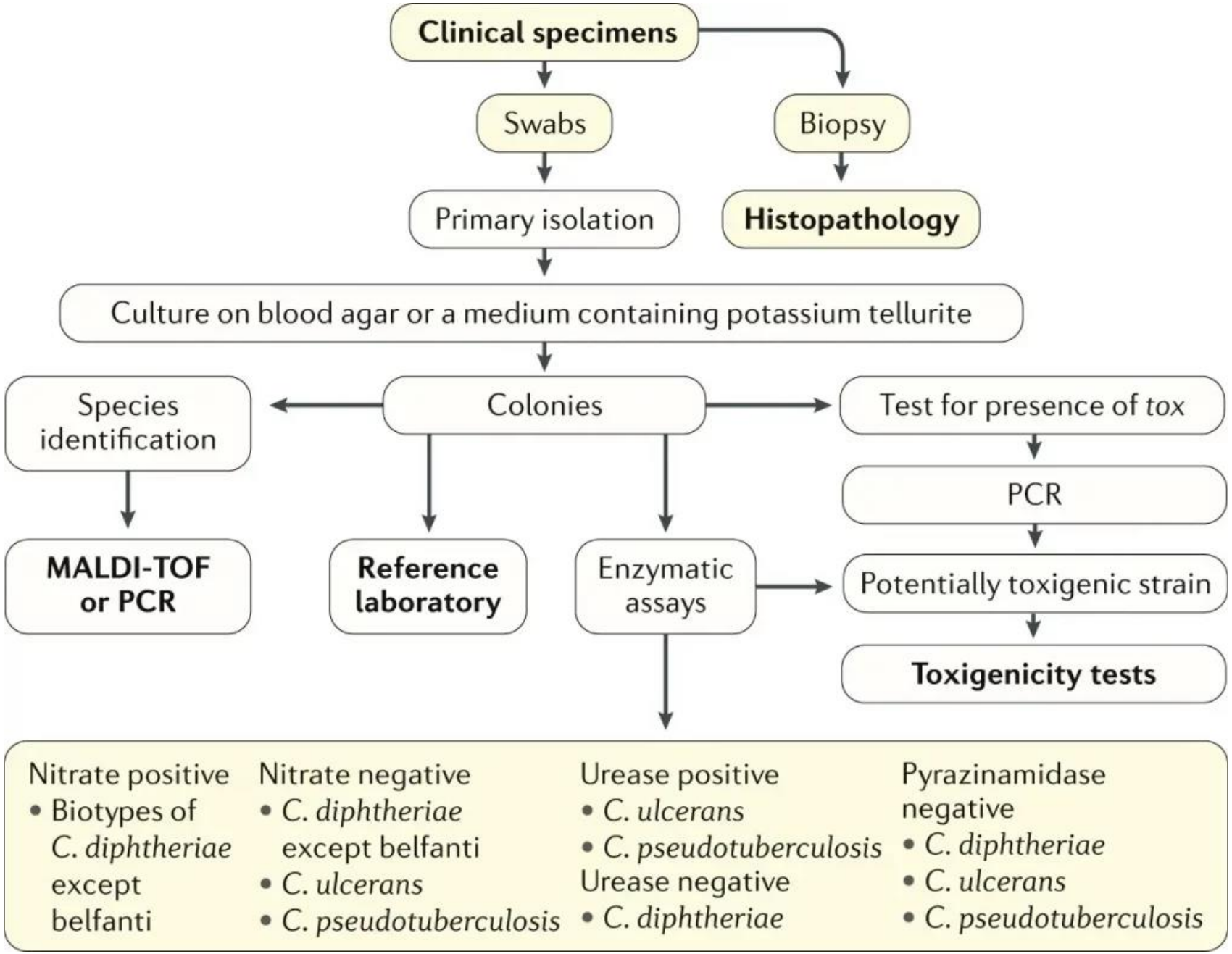


COMPLICATIONS

- Asphyxia : due to mechanical obstruction
  - ➢ Emergency tracheostomy may be necessary;
- Acute circulatory failure
- Myocarditis
- Postdiphtheritic paralysis-
  - ➢ palatine(soft palate) and ciliary (eye muscles) nerves
  - ➢ Recovery – spontaneous and complete
- Septic : pneumonia and otitis media
- Relapse : in about 1% of cases

LABORATORY DIAGNOSIS

- Specimens :
  - Swabs from – nose, throat or other suspected lesions;
- Smear examination: Gram stain
  - shows beaded rods in typical arrangement;
  - Difficult to differentiate from some commensal corynebacteria normally found in throat;
  - Albert's stain or Neisser's stain is useful for demonstrating the granules;

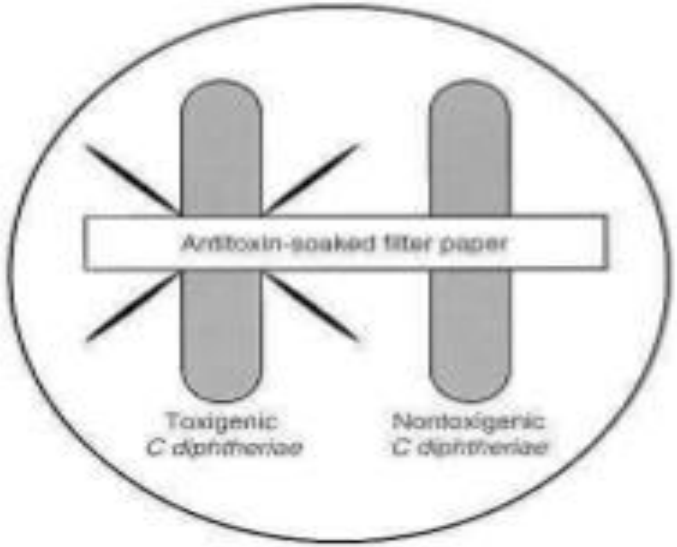

Clinical specimens
Swabs
Biopsy
Primary isolation
Histopathology
Culture on blood agar or a medium containing potassium tellurite
Species identification
Colonies
Test for presence of tox
PCR
MALDI-TOF or PCR
Reference laboratory
Enzymatic assays
Potentially toxigenic strain
Toxigenicity tests
Nitrate positive
• Biotypes of C. diphtheriae except belfanti
Nitrate negative
• C. diphtheriae except belfanti
• C. ulcerans
• C. pseudotuberculosis
Urease positive
• C. ulcerans
• C. pseudotuberculosis
Urease negative
• C. diphtheriae
Pyrazinamidase negative
• C. diphtheriae
• C. ulcerans
• C. pseudotuberculosis

LABORATORY DIAGNOSIS : CULTURE

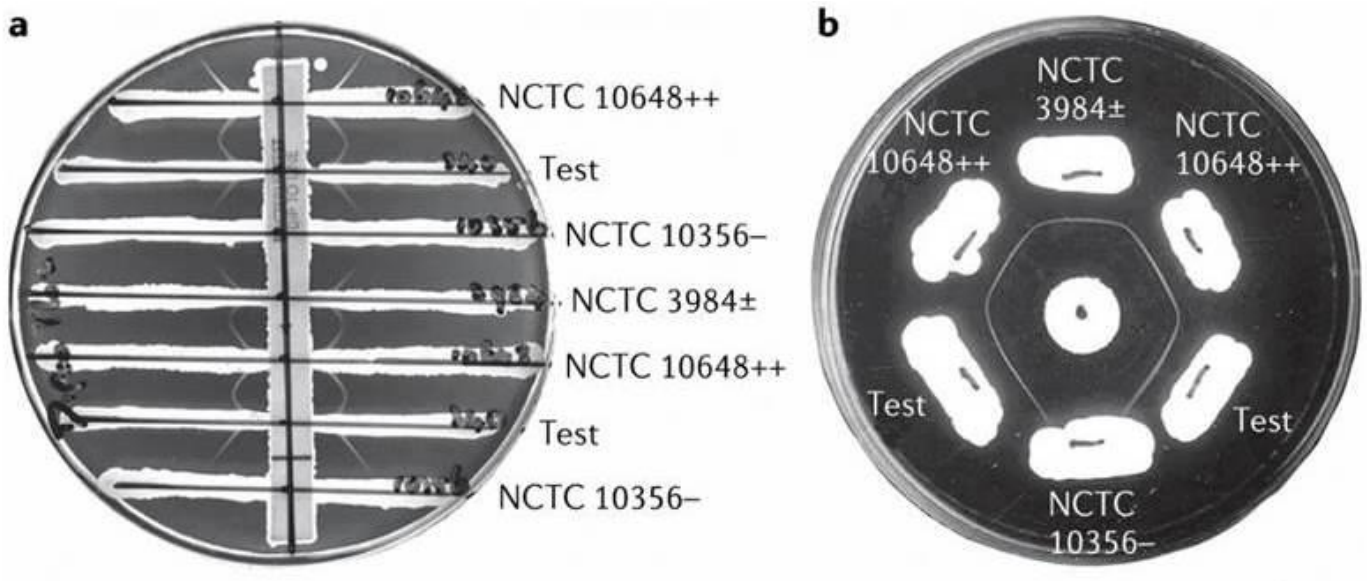
- If the swabs can not be inoculated promptly, they should be kept moistened with serum;
- Inoculate on :
  - **Loeffler's serum slope**
  - **Tellurite blood agar or Tinsdale medium**
  - **Blood agar (for differentiating Staphylococcal or Streptococcal pharyngitis that simulate diphtheria);**
- *Tellurite medium is particulary useful for isolating the organism from – convalescents, contacts or carriers;*

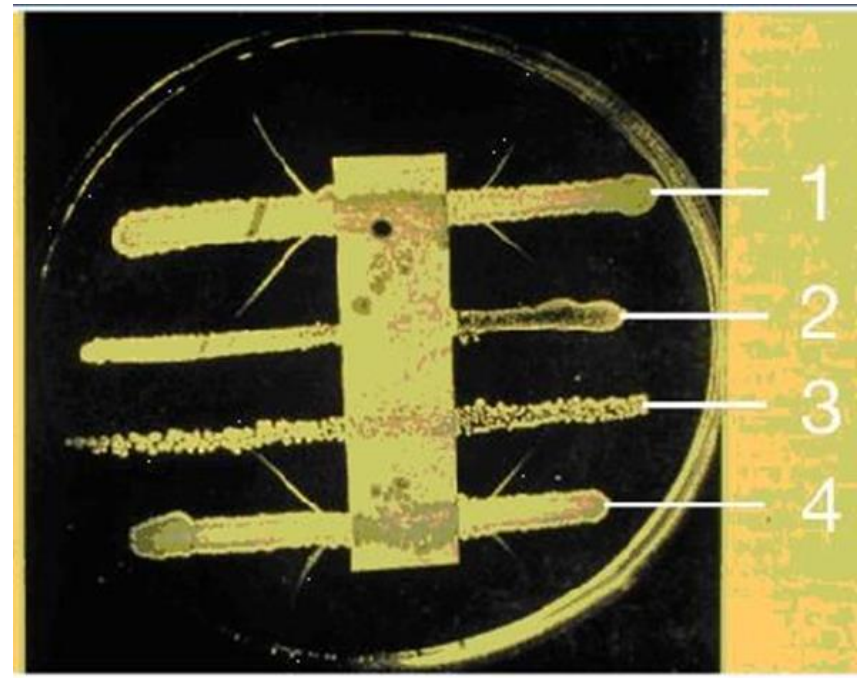
Elek immunodiffusion test

- A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and *C diphtheriae* isolates are streak-inoculated at a 90° angle to the filter paper.
- The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.



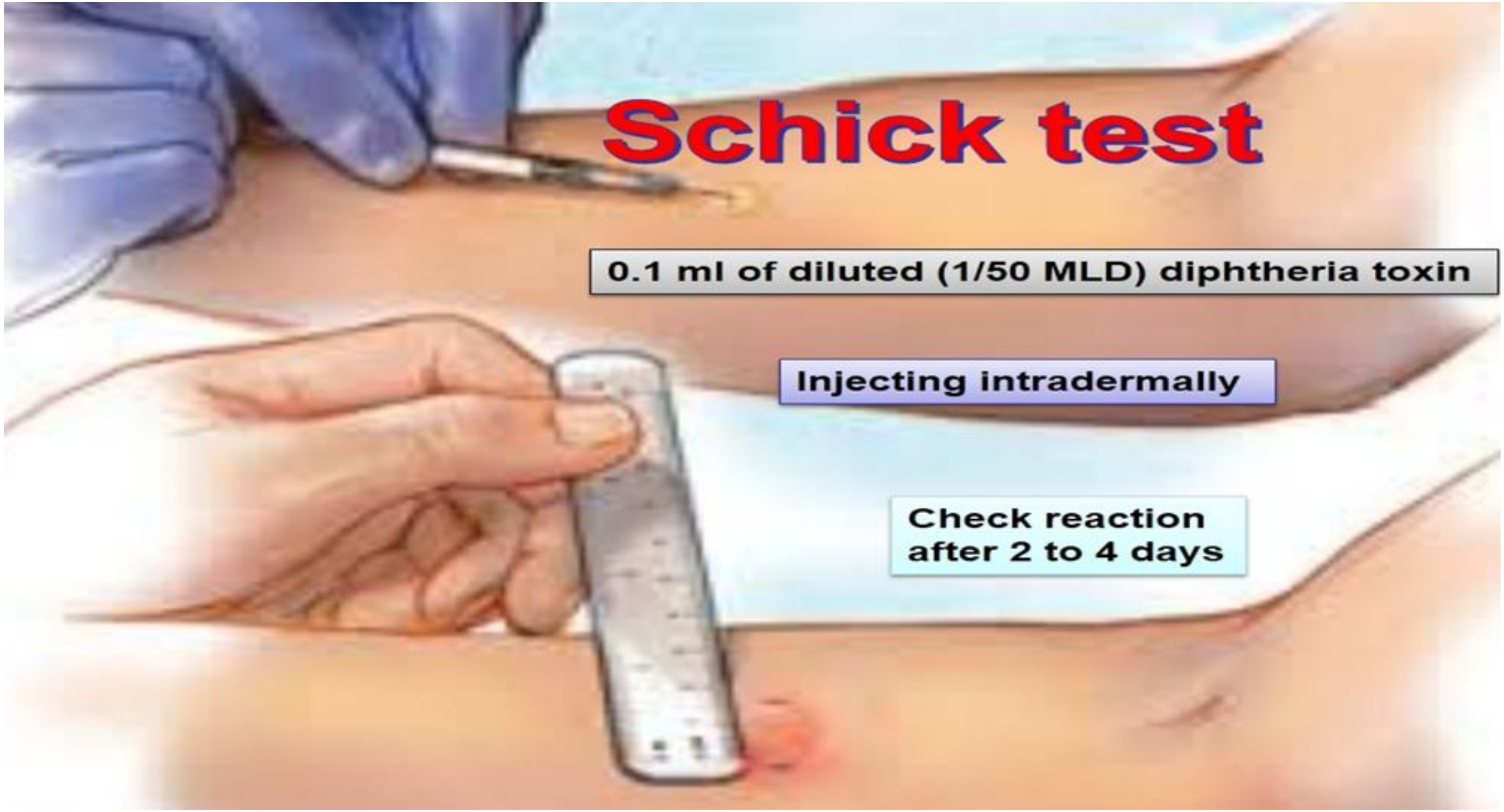
Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheriae* is detected because secreted toxin diffuses from the area of growth and reacts with antitoxin to form lines of precipitin.


a
NCTC 10648++
Test
NCTC 10356–
NCTC 3984±
NCTC 10648++
Test
NCTC 10356–
b
NCTC 3984±
NCTC 10648++
NCTC 10648++
Test
Test
NCTC 10356–


1
2
3
4

Schick test

- A small amount (0.1 ml) of diluted (1/50) diphtheria toxin is injected intradermally into the arm of the person. If a person does not have enough antibodies to fight it off, the skin around the injection will become red and swollen, indicating a positive result. If the person has an immunity, then little or no swelling and redness will occur, indicating a negative result.


Schick test
0.1 ml of diluted (1/50 MLD) diphtheria toxin
Injecting intradermally
Check reaction
after 2 to 4 days

Interpretation

- Negative reaction: If a person had immunity to diphtheria, no reaction will be observed on either arm.

- Positive reaction: An area of in duration 10-15 mm in diameter generally appears within 24-36 hours reaching its maximum development by 4-7 days, the control arm shows no change. The person is susceptible to diphtheria.

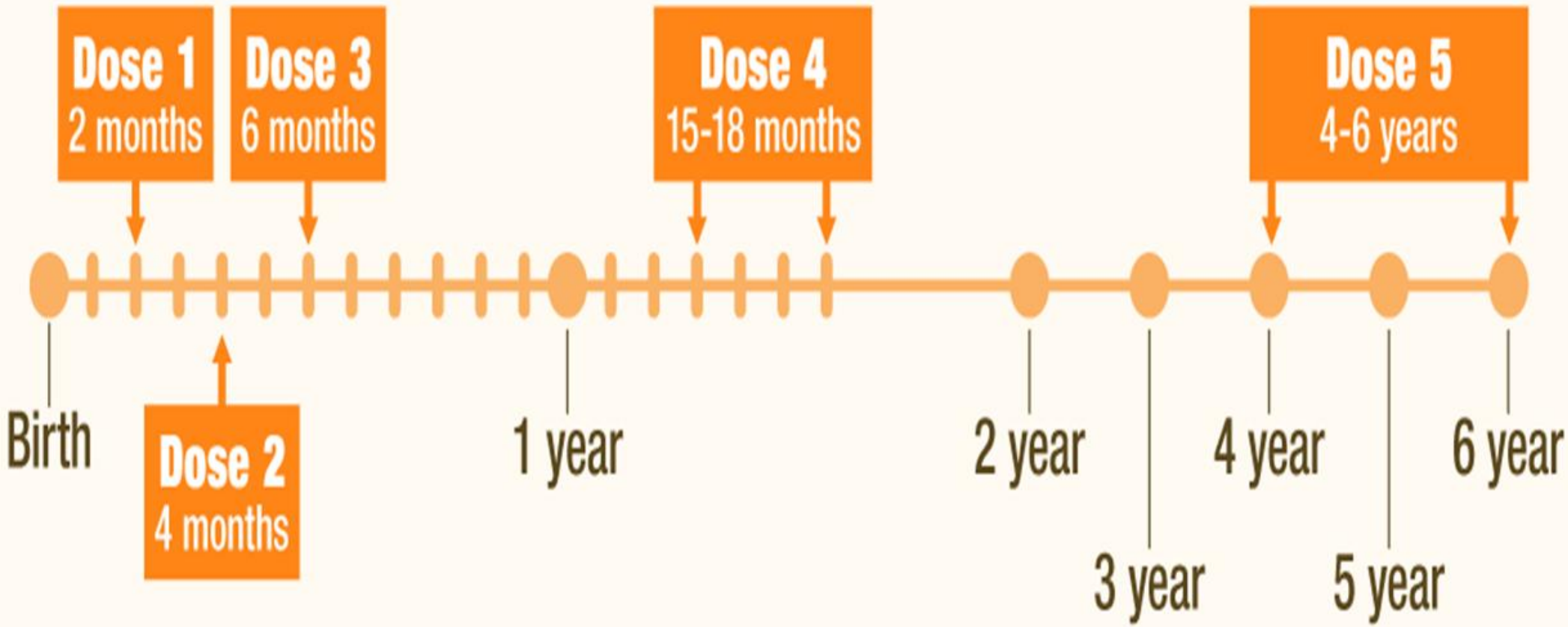
- False positive reaction: A red flush develops in both arms, the reaction fades very quickly, and disappears by 4th day. This is an allergic type of reaction found in certain individuals
- Combined reaction: the control arm shows pseudo positive reaction and the test arm is true +ve reaction, susceptible and need vaccination

Prevention

Vaccination: Immunisation with diphtheria toxoid, combined with tetanus and pertussis toxoid (DTP vaccine), should be given to all children at two, three and four months of age. Booster doses are given between the ages of 3 and 5 .

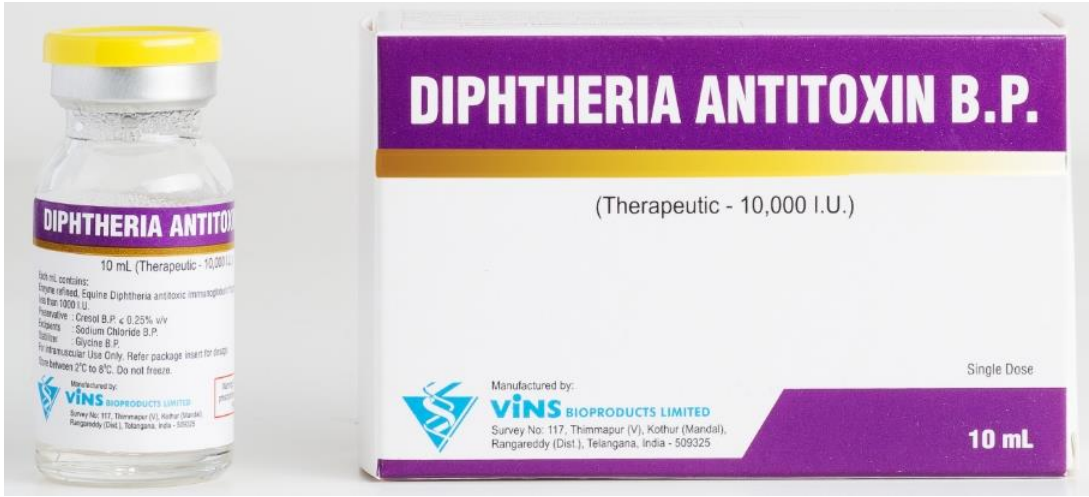
The child is given a further booster vaccine before leaving school and is then considered to be protected for a further 10 years (16 – 18 years).

DTaP: Diphtheria, Tetanus, and Acellular Pertussis



Treatment

- Antibiotic not useful in Acute infections,
- Antitoxin a must.
- Anti toxin obtained from horse serum
- Mild 20,000 to 40,000
- Moderate 40,000 to 60,000
- Severe 80,000 to 1,00,000
- Commonly used antibiotics,
- Penicillin parentally,
- Oral Erythromycin

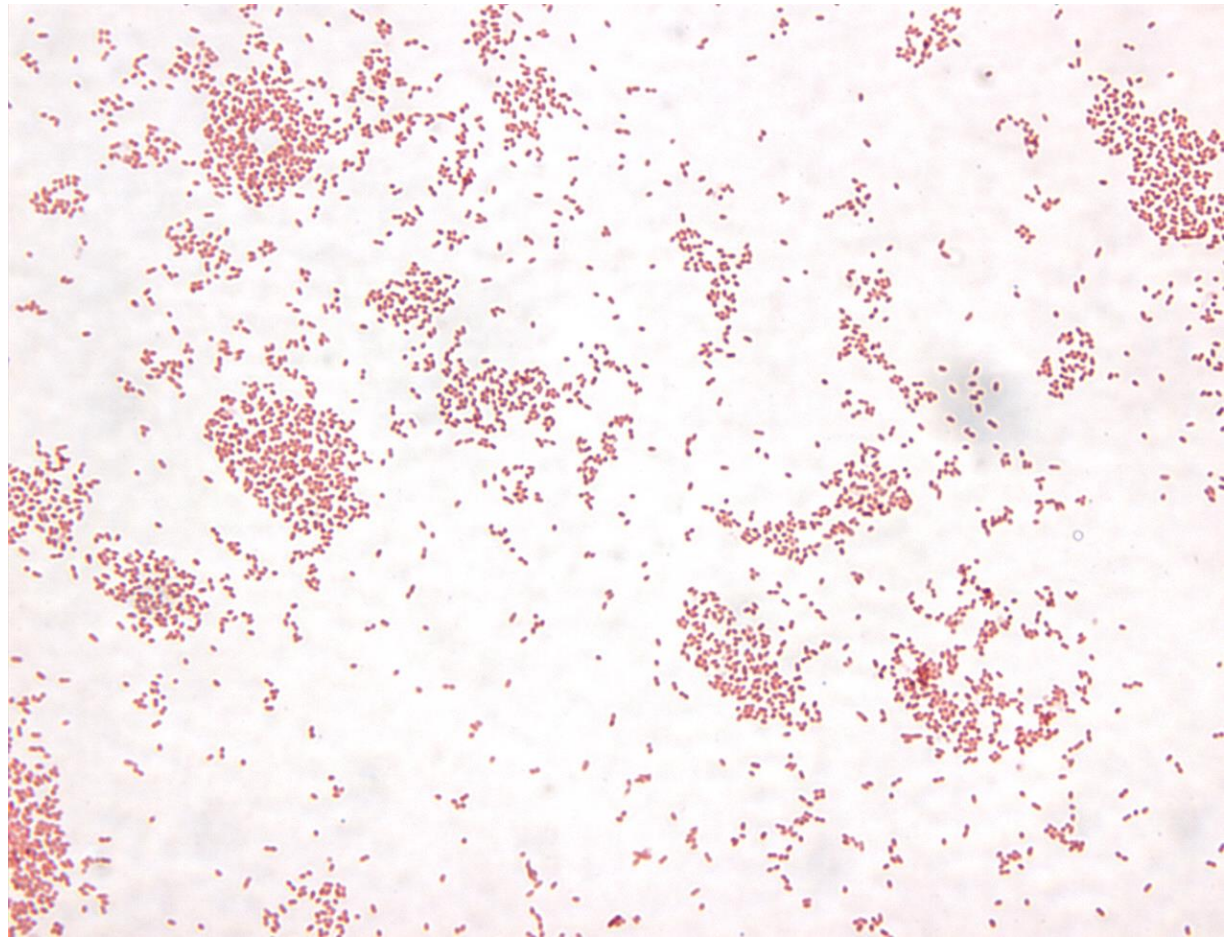


Bordetella - Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Betaproteobacteria
- (Order): Burkholderiales
- (Family): Alcaligenaceae
- (Genus): Bordetella
- (Species): ***B.pertussis, B.parapertussis,*** *B.bronchiseptica, B.avium etc.*

Morphology

- It is Gram negative.
- It is a small, ovoid coccobacillus (mean length 0.5 µm).
- It is nonmotile and nonsporing.
- It is capsulated.
- Freshly isolated strains of Bord pertussis have fimbriae.

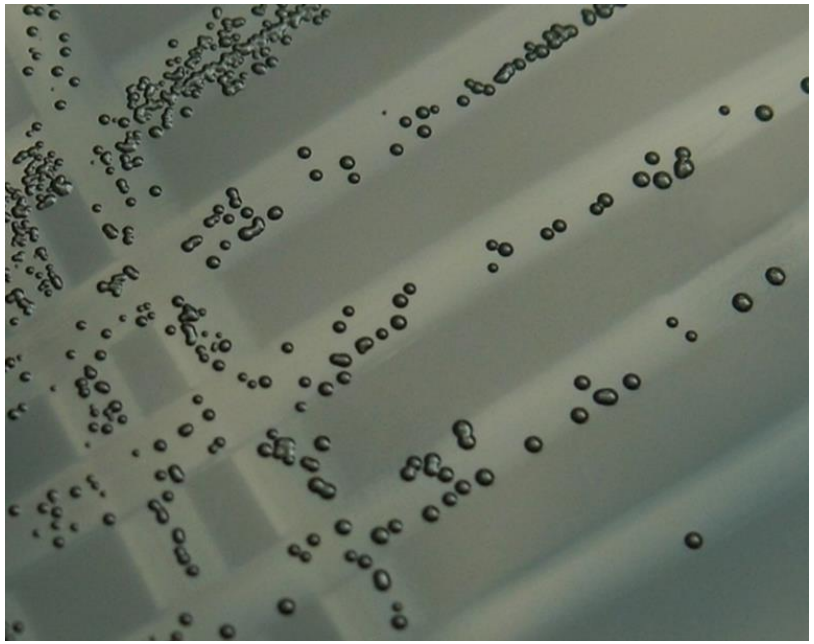


Cultural characteristics

- Aerobic, Not anaerobic
- Grows optimally at 35^0 to 37^0 c
- Complex media are necessary for primary isolation
- Preferred medium – **Bordet Gengou glycerin potato blood agar**
- Blood for neutralizing inhibitory substances formed during bacterial growth.
- Charcoal also serves the same purpose.
- Charcoal blood agar is a useful medium.
- It does not grow on, simple media like nutrient agar.

Colonies on Bordet-Gengou medium

- Growth is slow(48-72 hours).
- Colonies are small, dome-shaped, smooth, opaque, viscid, greyish white, refractile and glistening, resembling 'bisected pearls' or 'mercury drops'.
- Surrounded by a hazy zone of hemolysis.
- Confluent growth presents an 'aluminium paint' appearance.

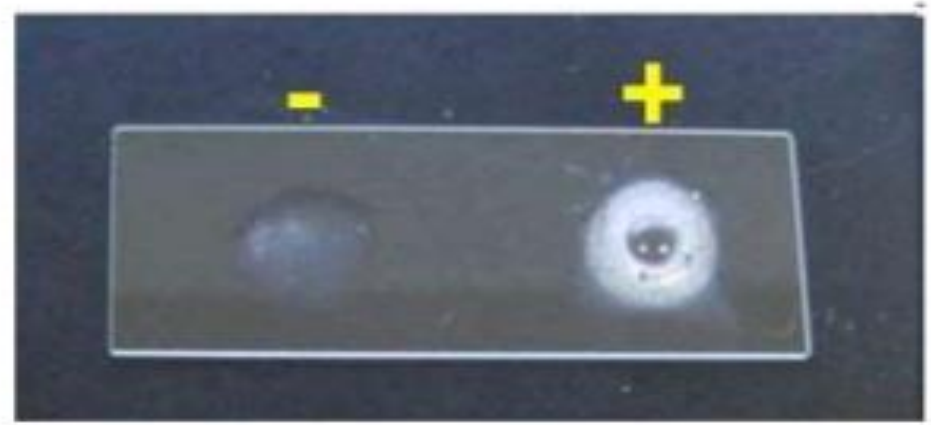


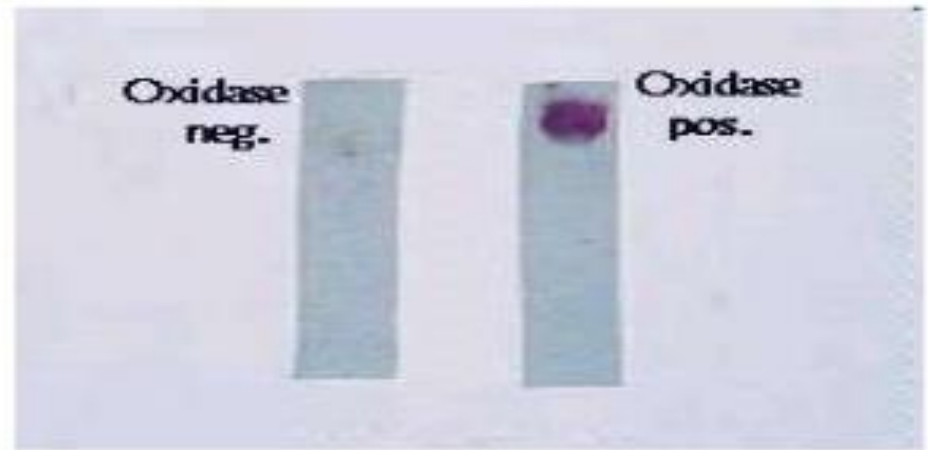
Differentiating features of Bordetella species

	B.pertusis	B.Parapert - usis	B.bronchis eptila	B.canium
Motality	-	-	+	+
Growth on nutrient agar	-	+	+	+
Growth B-G medium	3 - 6	1 -2	1	1
urease	-	+	+	-
Nitrate to nitrate	-	-	+	-

Biochemical reactions

- Do not ferment sugars
- Indole test +
- Nitrates +
- Citrates +
- Urease +
- **Catalase +**
- **Oxidase +**



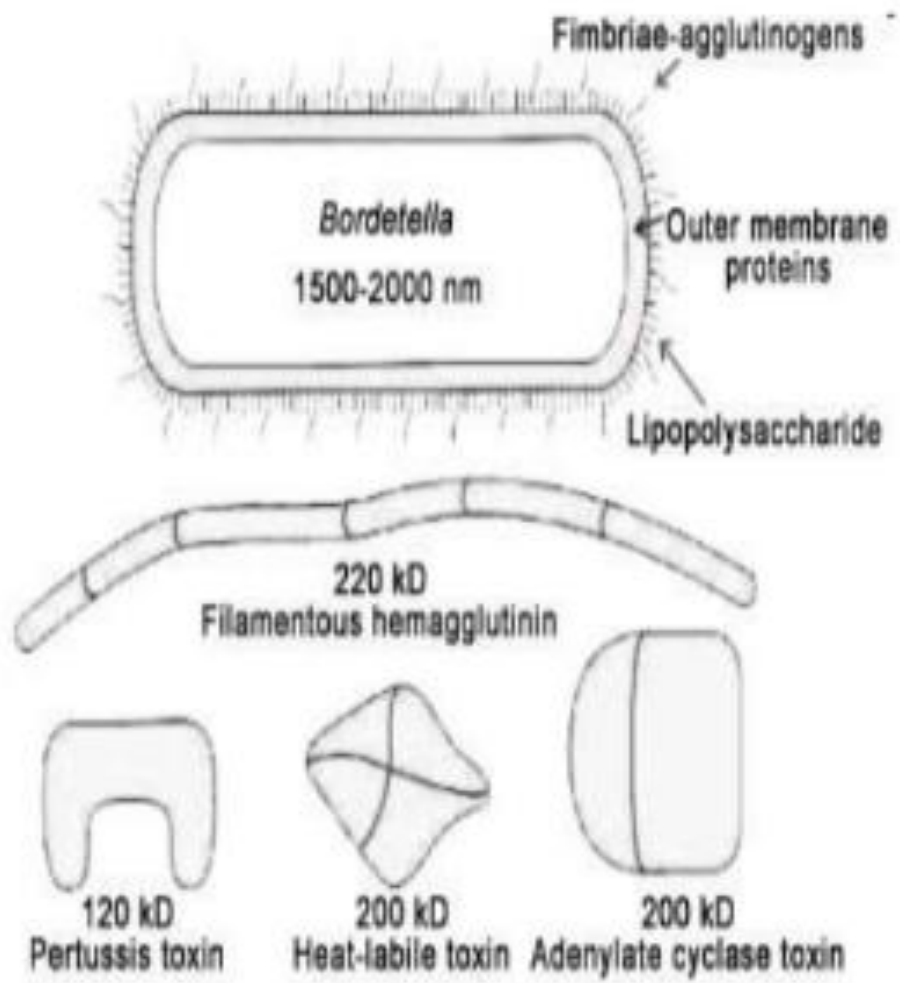


Resistance

- It is a delicate organism, being killed readily by heat (55°C for 30 minutes), drying and disinfectants.
- Outside the body, Bord pertussis in dried droplets is said to survive for five days on glass, three days on cloth and a few hours on paper.

Antigenic constituents and virulence factors

- Several antigenic fractions and putative virulence factors have been described but their role in the pathogenesis of pertussis remains to be clarified.
- These virulence factors include:
- Adhesions such as filamentous hemagglutinin, agglutinogens, peractin, and fimbriae.
- A number of toxins including pertussis toxin, acetylate cyclase toxin, trachael cytotoxins, Dermonecrtoic toxin and heat-labile toxin.



Agglutinogens

- Species specific surface agglutinogens with capsule K antigens or fimbria
- 14 agglutinin factors are identified
- Factors 7 is common in all species
- Factor 1- 6 in only B pertussis
- Factor 12 in B.brochoseptica
- Factor 14 in B parapertussis
- Agglutinogens promote virulence by helping bacteria to attach to respiratory epithelial cells.
- They are useful in serotyping strains and in epidemiological studies.

TABLE 39-2 Virulence factors of *Bordetella pertussis*

Virulence factors	Biological functions
Filamentous hemagglutinin	Binds to galactose residues on a sulfated glycolipid called sulfatide, present on the surface of the ciliated cells; binds to CR3, a receptor on the surface of polymorphonuclear leukocytes; and mediates the attachment of *Bordetella pertussis* to ciliated epithelial cells of the respiratory tract
Pertussis toxin	Causes adhesion of *Bordetella pertussis* to tracheal epithelium; S2 subunit binds to glycolipid present on ciliated epithelium; S3 subunit binds to ganglioside receptor on the surface of phagocytic cells; and S1 subunit inhibits the eukaryotic adenyl cyclase, killing of phagocytes, and migration of monocytes
Invasive adenylate cyclase or hemolysin	It has adenylate cyclase activity and a binding component that mediates attachment to host cell surface
Lethal toxin	Causes inflammation and lethal necrosis around the site of adherence of the bacteria
Tracheal cytotoxin	Kills ciliated respiratory cells; also stimulates release of cytokine IL-1 and inhibits ciliary movement

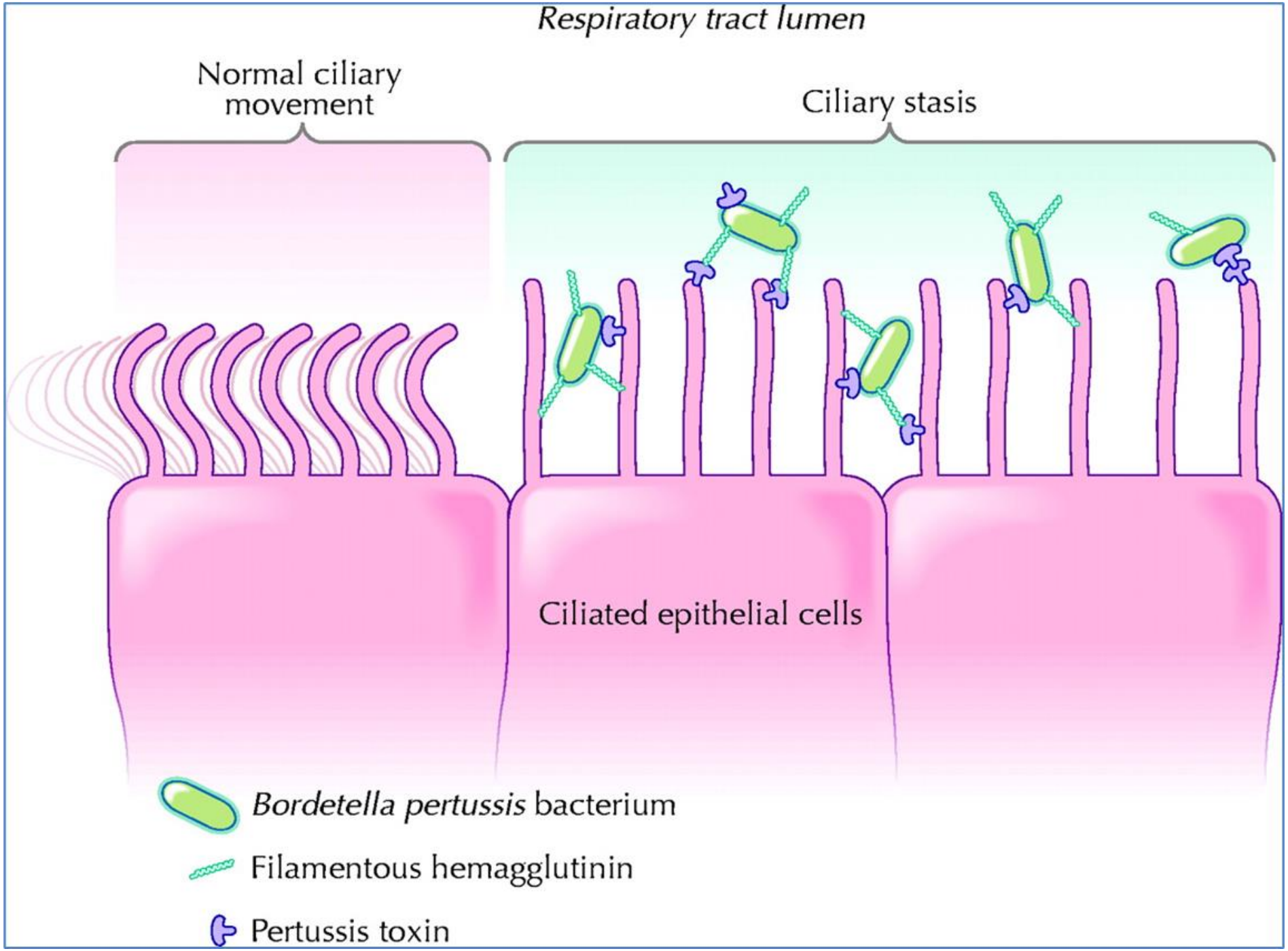
Pertussis toxin (PT)

- This is present **only in Bord pertussis**. It plays an important role in the pathogenesis of **whooping cough**.
- PT is expressed on the surface of the bacillus and secreted into the surrounding medium
- The toxin exhibits diverse biological and biochemical activities, which formerly had been believed to be caused by different substances that had been named accordingly.
- Examples are the lymphocytosis producing factor(LPF), causing profound lymphocytosis in pertussis patients as well as in experimental animals, and two effects seen only in experimental animals but not in patients,

Cont.....

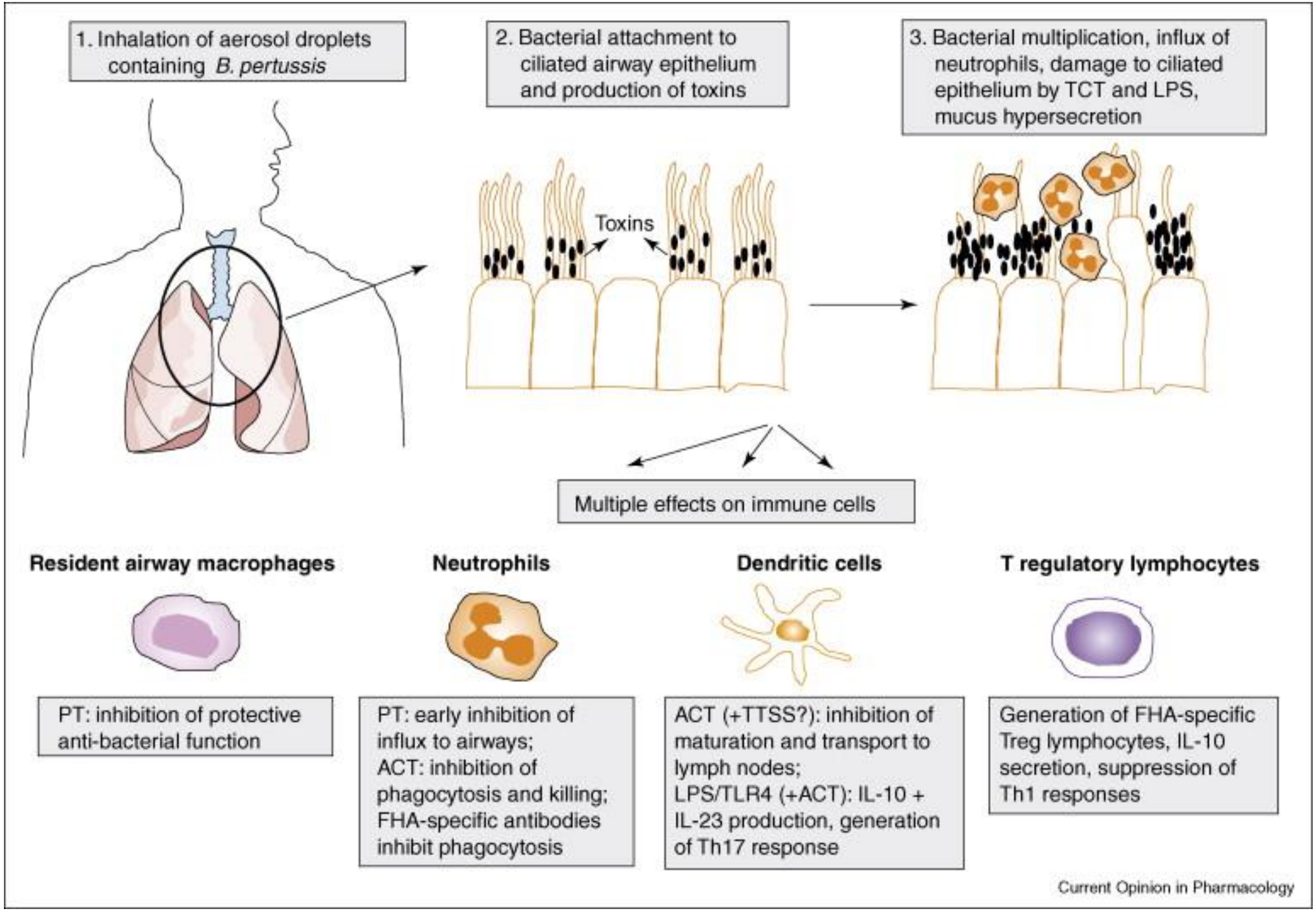
Pertussis toxin (PT)

- Such as the histamine sensitising factor(HSF) responsible for heightened sensitivity to histamine in experimental animals, and the islet activating protein(IAP) inducing excessive insulin secretion by the pancreatic islet cells.
- It is now known that all these are manifestations of the pertussis toxin
- PT is a 117,000 molecular weight hexamer protein composed of six subunits with an A-B structure (the A portion being the enzymatically active moiety and B the binding component).
- **It can be toxoided**.
- PT toxoid is the major component of acellular pertussis vaccines

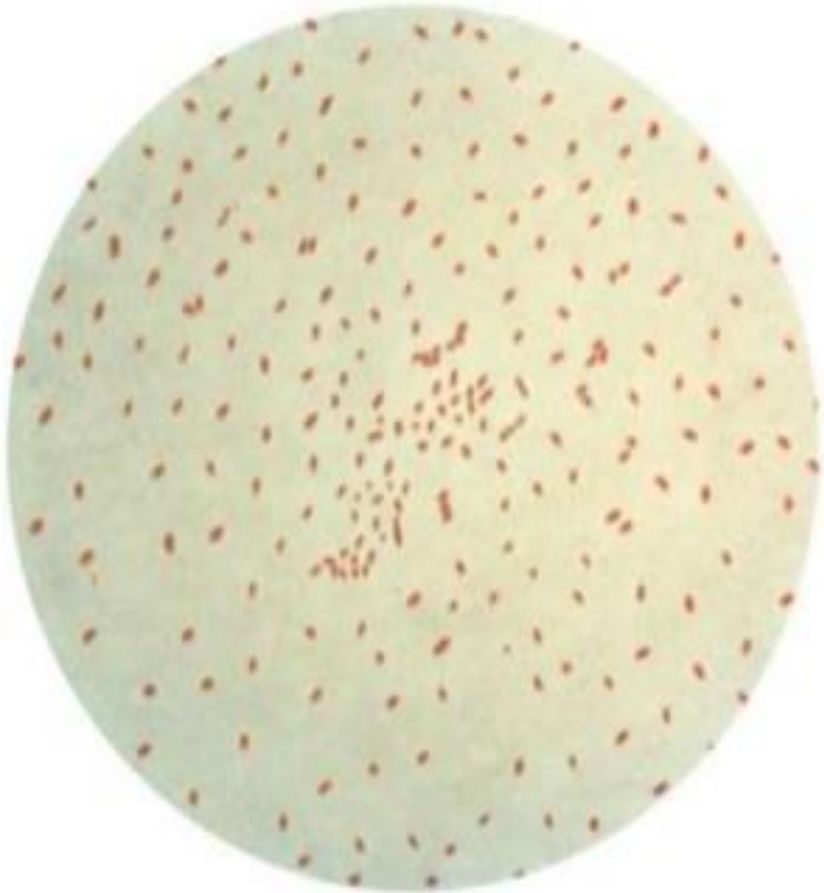

Respiratory tract lumen
Normal ciliary movement
Ciliary stasis
Ciliated epithelial cells
Bordetella pertussis bacterium
Filamentous hemagglutinin
Pertussis toxin

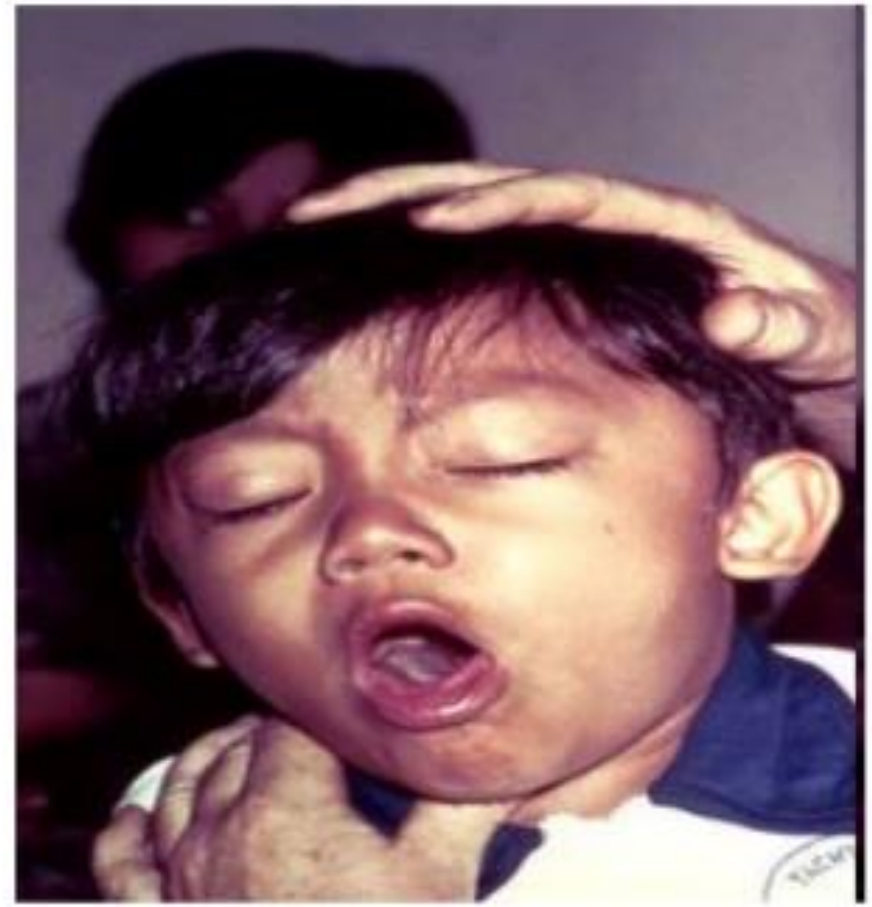
Pathogenicity & Clinical Manifestations

- In human beings, after an incubation period of about 1-2 weeks, the disease takes a protracted course comprising three stages:
- the catarrhal,
- paroxysmal and
- convalescent each lasting approximately two weeks.
- The onset is insidious, with low grade fever, catarrhal symptoms and a dry, irritating cough.
- Clinical diagnosis in the catarrhal stage is difficult.
- This is unfortunate as this is the stage at which the disease can be arrested by antibiotic treatment.


1. Inhalation of aerosol droplets containing *B. pertussis*
2. Bacterial attachment to ciliated airway epithelium and production of toxins
3. Bacterial multiplication, influx of neutrophils, damage to ciliated epithelium by TCT and LPS, mucus hypersecretion
Toxins
Multiple effects on immune cells
Resident airway macrophages
Neutrophils
Dendritic cells
T regulatory lymphocytes
PT: inhibition of protective anti-bacterial function
PT: early inhibition of influx to airways; ACT: inhibition of phagocytosis and killing; FHA-specific antibodies inhibit phagocytosis
ACT (+TTSS?): inhibition of maturation and transport to lymph nodes; LPS/TLR4 (+ACT): IL-10 + IL-23 production, generation of Th17 response
Generation of FHA-specific Treg lymphocytes, IL-10 secretion, suppression of Th1 responses
Current Opinion in Pharmacology

Bordetella





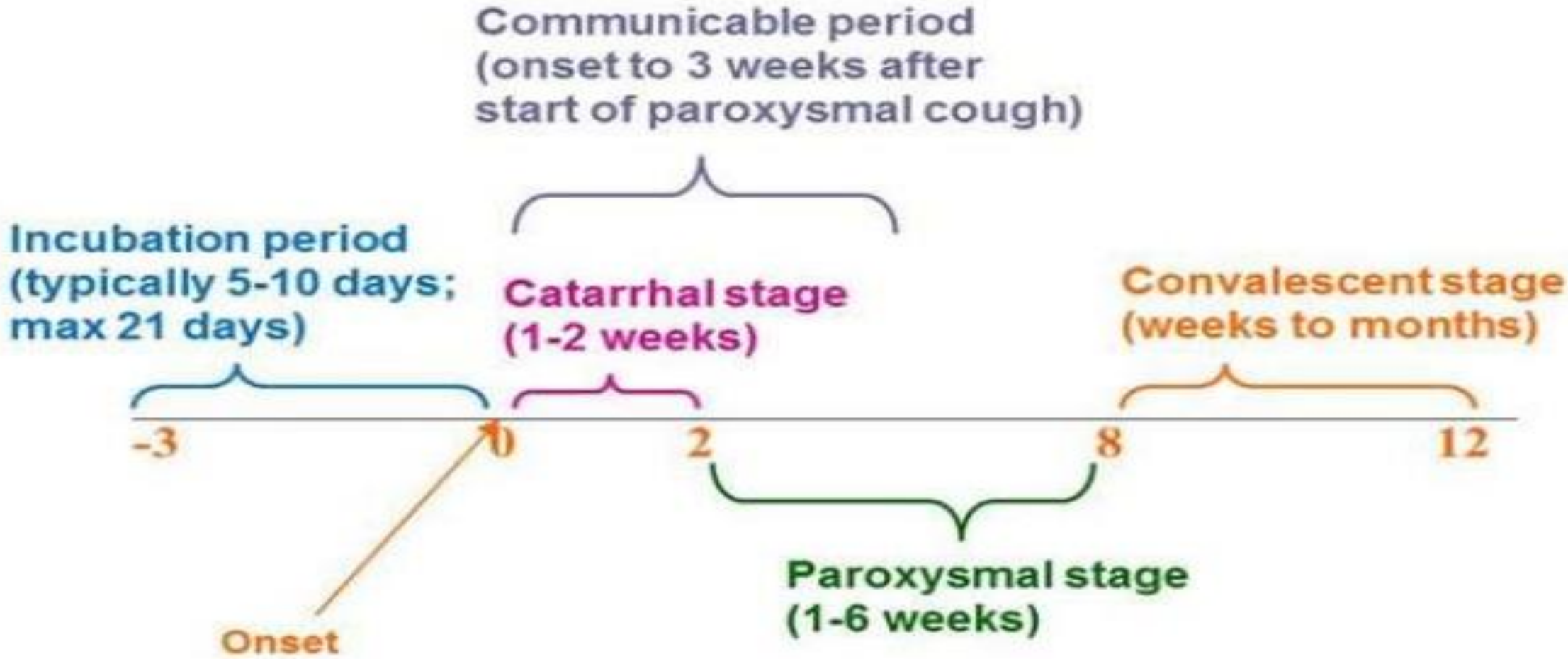
Clinical Manifestations of Pertussis

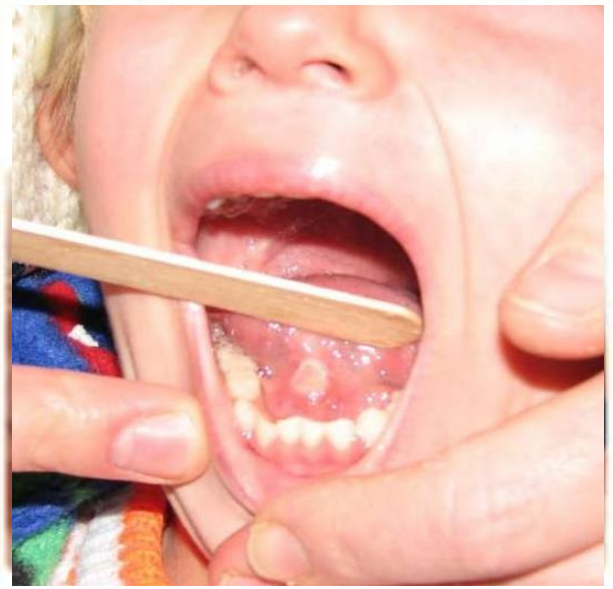
- Incubation period 3-12 days (up to 21 days)
- Insidious onset, similar to minor upper respiratory infection with nonspecific cough
- Fever usually minimal throughout course
- Apnea & Cyanosis in infant

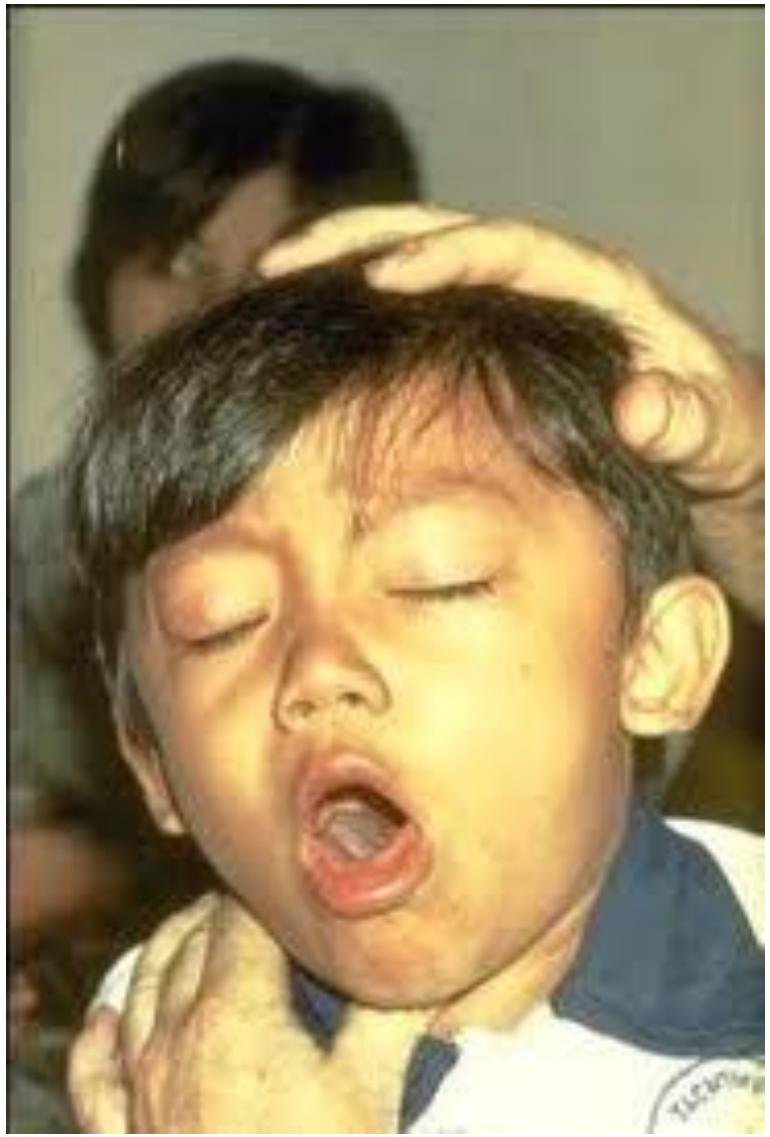
STAGES

- 1st Stage- Catarrhal Stage
- 2nd Stage- Paroxysmal Stage
- 3rd Stage- Covalescent Stage

Clinical Course (in weeks)

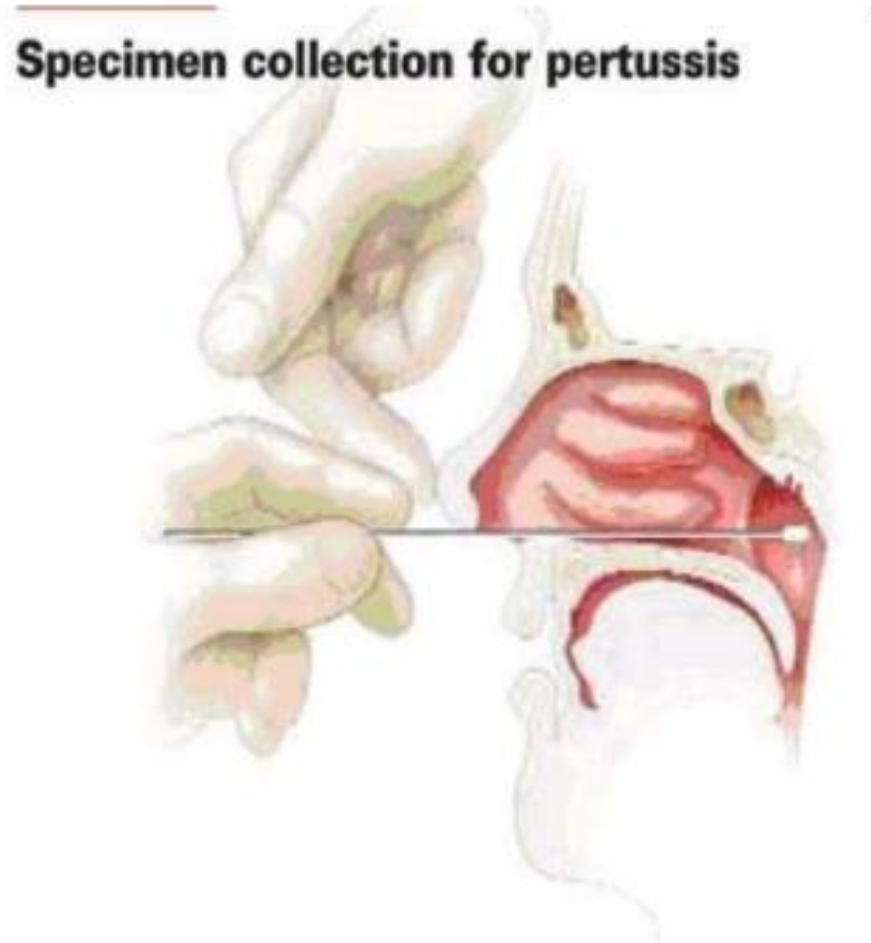






Nasopharyngeal Swab

- Secretion from the posterior pharyngeal wall are collected with cotton swab on a bent wire passed from the oral cavity
- A West's post nasal swab is used for collection of specimen.



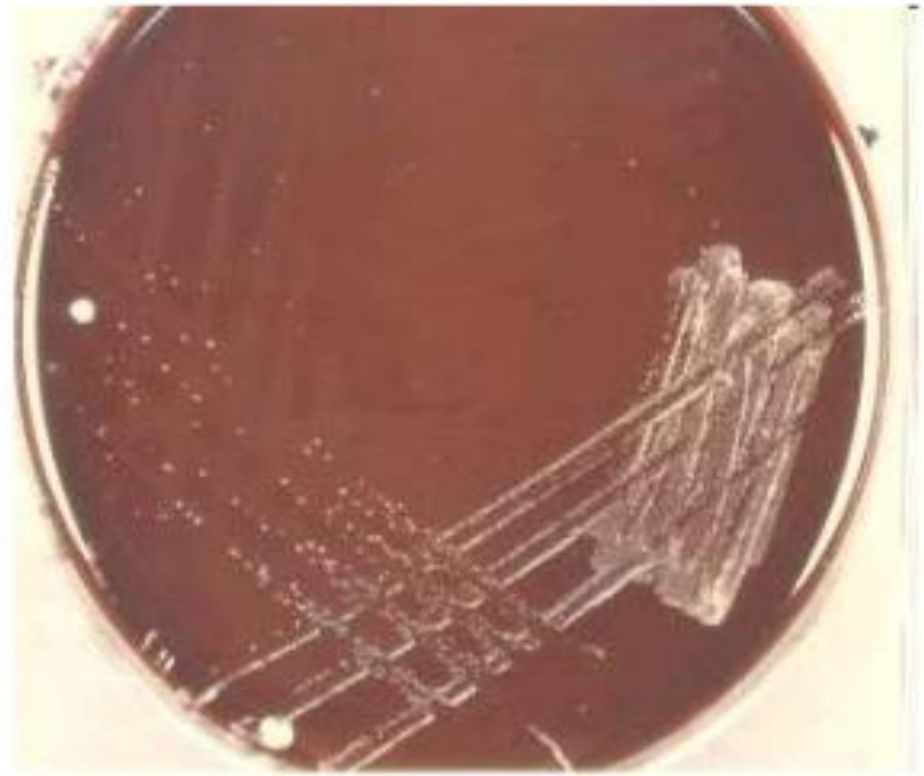
Laboratory Diagnosis

- Microscopy – Demonstration of Bacilli in respiratory secretions.
- Florescent Antibody methods.
- Specimen Collection:
- Cough Plate Method:
- Culture plate held at 10-15 cm infront of the mouth when the patient is coughing spontaneously or induced cough
- Droplets of respiratory exhaled impinge on the media.
- Helpful as bed side investigation

Cough Plate Method

Identification of bacteria

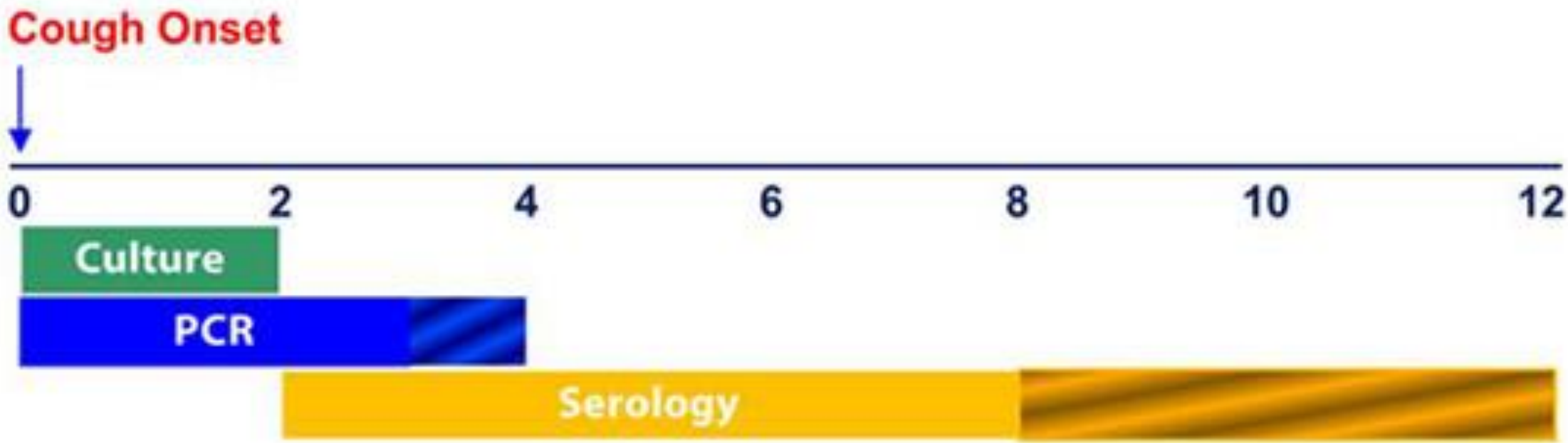
- The culture plates are incubated at 36^{0}c
- The bacteria are identified by Microscopy and slide agglutination
- Immunofluorescence methods



Serology

- Paired serum sample for detection of antibodies
- Gel precipitation testing
- Complement fixation test
- Detection of Ig A by ELISA from nasopharyngeal secretions.

Optimal Timing for Diagnostic Testing (weeks)



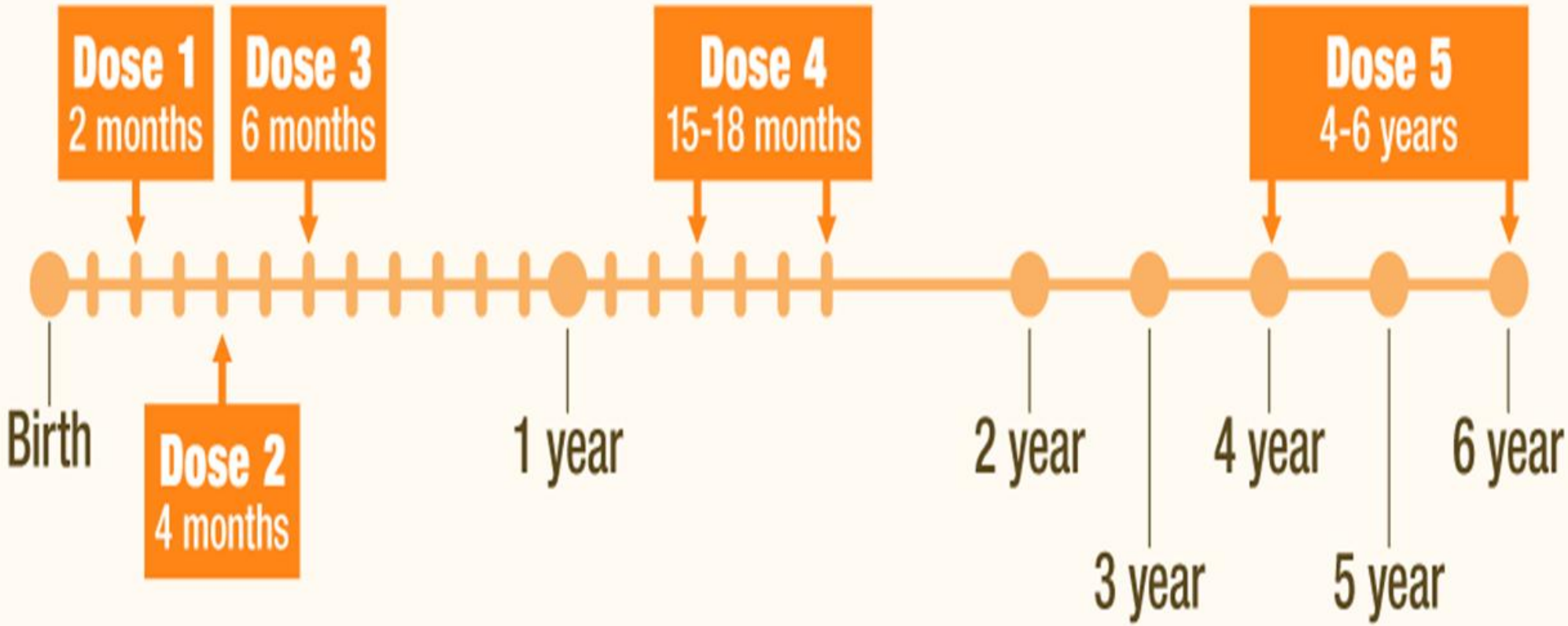
Choice of regimen

- Erythromycin
- Azithromycin
- Clarithromycin

Prophylaxis

- Preventing the spread of infection by isolation of cases is seldom practicable, as infectivity is highest in the earliest stage of the disease when clinical diagnosis is not easy.
- Specific immunisation with killed Bord pertussis vaccine has been found very effective.
- The alum absorbed vaccine produces better and more sustained protection and less reaction than the plain vaccines.
- Pertussis vaccine is usually administered in combination with diphtheria and tetanus toxoid (triple vaccine)(DTwP/DTaP).
- Not only is this more convenient but Bord pertussis also acts as an adjuvant for the toxoids, producing better antibody response.

DTaP: Diphtheria, Tetanus, and Acellular Pertussis



Haemophilus

Scientific classification

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Pasteurellales
Family: Pasteurellaceae
Genus: Haemophilus
Species: influenzae
Binomial name: *Haemophilus influenzae*

Haemophilus species of clinical importance

1. ***H. influenzae***

-**type b is an important human pathogen**

2. ***H. ducreyi***

-**sexually transmitted pathogen (chancroid)**

3. Other *Haemophilus are normal flora*

- *H. parainfluenzae – Pneumonia & endocarditis*
- *H. aphrophilus – Pneumonia & endocarditis*
- *H. aegyptius – Pink eye (purulent conjunctivitis)*

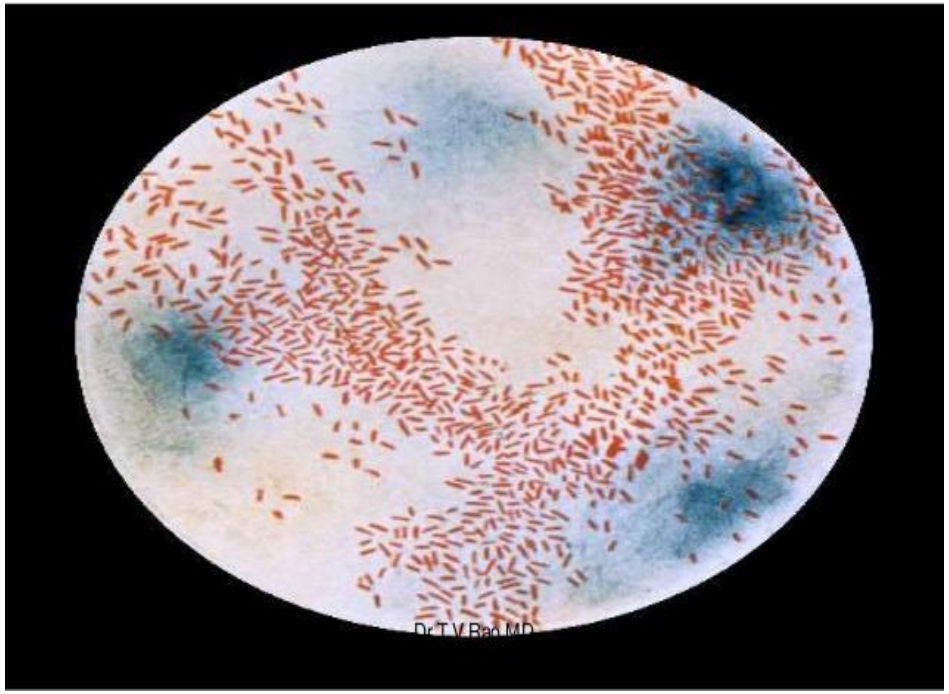
Haemophilus Influenza

- Aerobic gram-negative bacteria
- Polysaccharide capsule
- Six different serotypes (a-f) of polysaccharide capsule
- 95% of invasive disease caused by type b (Hib)

Morphology

- Size is (1-2 X 0.3 – 0.5 microns)
- Non motile,
- Non sporing
- Gram negative rod or coccobacillus
- Pleomorphic (old culture)
- Appear as clusters of Coccobacillary forms in infected Sputum
- Long bacillary and filamentous form in infected CSF (Meningitis)

Haemophilus influenza



Gram staining

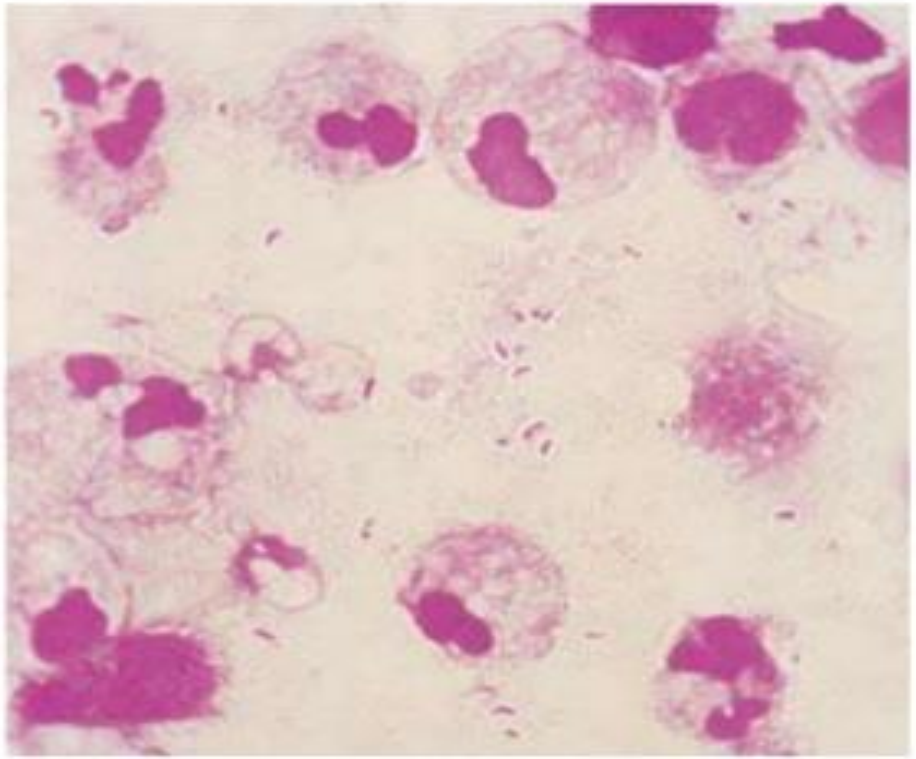


Fig: Gram-stained CSF sediment preparation.
Fine, Gram-negative rods surrounded by a capsule (serovar b).
Clinical diagnosis: purulent meningitis

Culture characteristics

- Fastidious growth requirements
- Factors X and V are essential for growth
- X is Hemin heat stable
- Porphyrins for synthesis of Cytochromes
- V-factor (NAD): Heat- labile, coenzyme I, nicotinamide adenine dinucleotide, found in blood – oxidation
- Aerobic 37 dg C

Haemophilus Species, cont.

Species	X	V	Hemolysis
►H. influenzae (H. aegyptius)	+	+	-
►H. parainfluenzae	-	+	-
►H. ducreyi	+	-	-
►H. haemolyticus	+	+	+
►H. parahaemolyticus	-	+	+
►H. aphrophilus	-	-	-

Culture characteristic

- On Chocolate agar, flat, grayish-brown colonies, 1-2 mm in diameter present after 24 hrs
- Colonies of staphylococci on sheep Blood agar cause the release of NAD, yielding satellite growth phenomenon

Satellite growth

When *Staph aureus* is streaked across plate of Blood agar with a species containing *H Influenzae*

Biochemical reaction

- Catalase +ve
- Oxidase +ve
- Reduces nitrite to nitrate
- Ferment glucose and galactose
- Can’t ferment sucrose, lactose and mannitol

Antigenic Properties

- Contains 3 Major surface antigens

1 Capsular polysaccharide

2 Outer membrane proteins (OMP)

3 Lipopolysaccharides (LPS)

Virulence factor of H. influnzae

- Polysaccharid capsule
- Fimbriae
- LPS- lipid A
- All virulence strain produce Neuraminidase (bioflim) and IgA protease.
- No exotoxin

Haemophilus Influenza

Mode of Transmission:

- Droplet infection and discharge from the upper
- respiratory tract during the infectious period.

Incubation Period

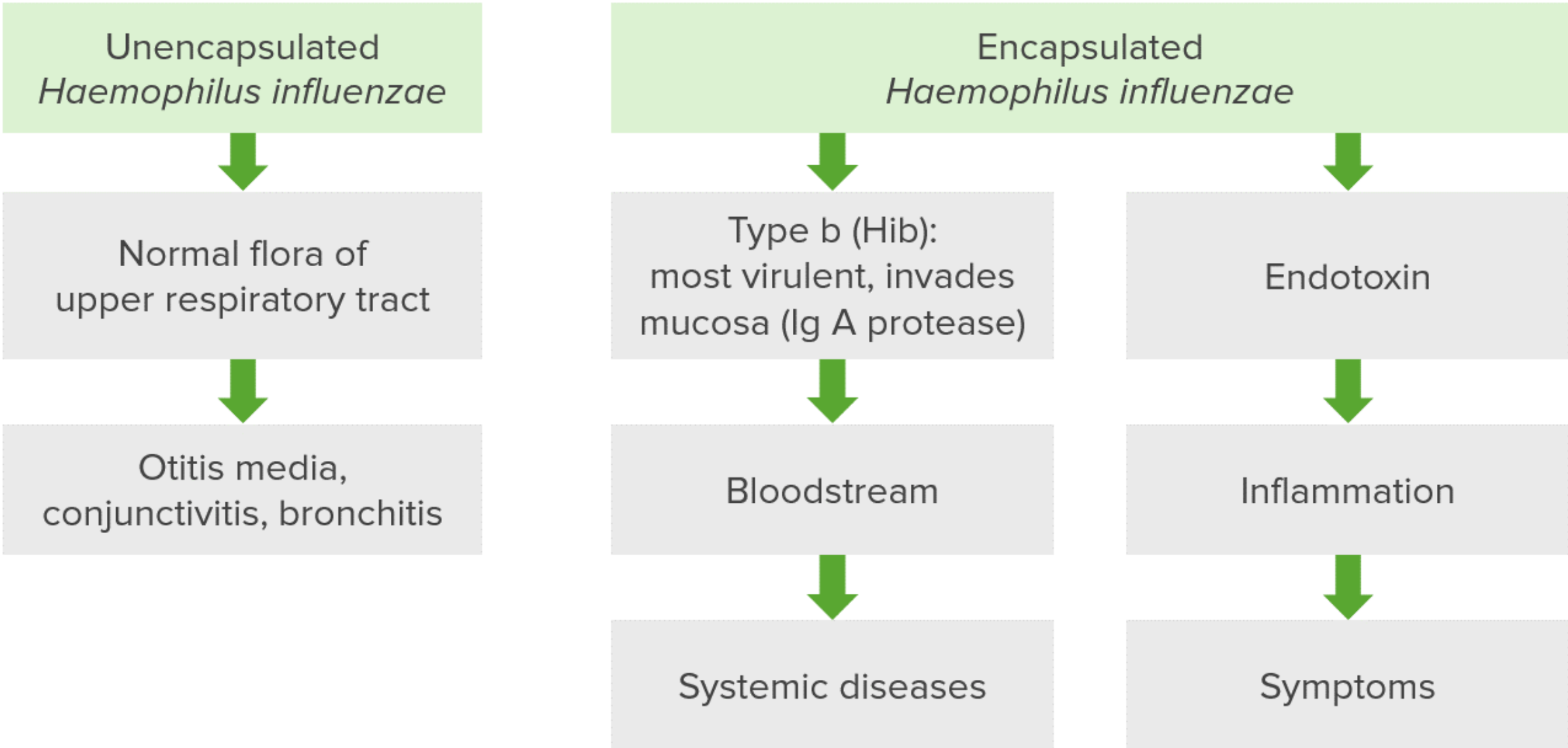
- Unknown, probably short, 2-4 days.

Infectious Period

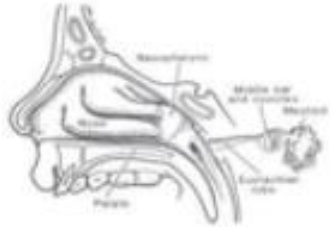
- - As long as the organism is present, even in the absence of nasal discharge.
- - Noninfectious within 24 to 48 hours after the start of effective antibiotics

Pathogenesis

- Type b *H influenzae* colonizes the nasopharynx, and may penetrate the epithelium and capillary endothelium to cause bacteremia
- Meningitis may result from direct spread via lymphatic drainage or from hematogenous spread.
- Nontypable *H influenzae* colonizes the nasopharynx and, to a lesser extent, the trachea and bronchi and may infect mucosa damaged by viral disease.
- Lipooligosaccharide is largely responsible for inflammation

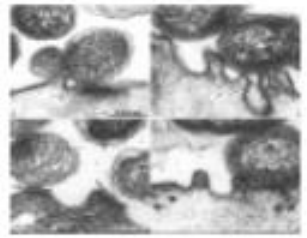

Unencapsulated
Haemophilus influenzae
Normal flora of
upper respiratory tract
Otitis media,
conjunctivitis, bronchitis
Encapsulated
Haemophilus influenzae
Type b (Hib):
most virulent, invades
mucosa (Ig A protease)
Bloodstream
Systemic diseases
Endotoxin
Inflammation
Symptoms

H. influenzae Pathogenesis

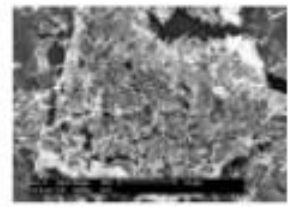


Transmission by droplet or contact

Commensal



Adherence to mucosal surface



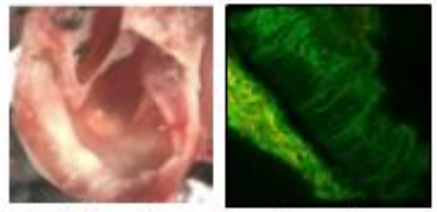
Replication/ colonization

Viral URT Infection

Ascension of virus-compromised Eustachian tube

Development of otitis media

Resolution of Disease



Biofilm formation in the middle ear

Clinical Presentation

Pneumonia

Septic Arthritis

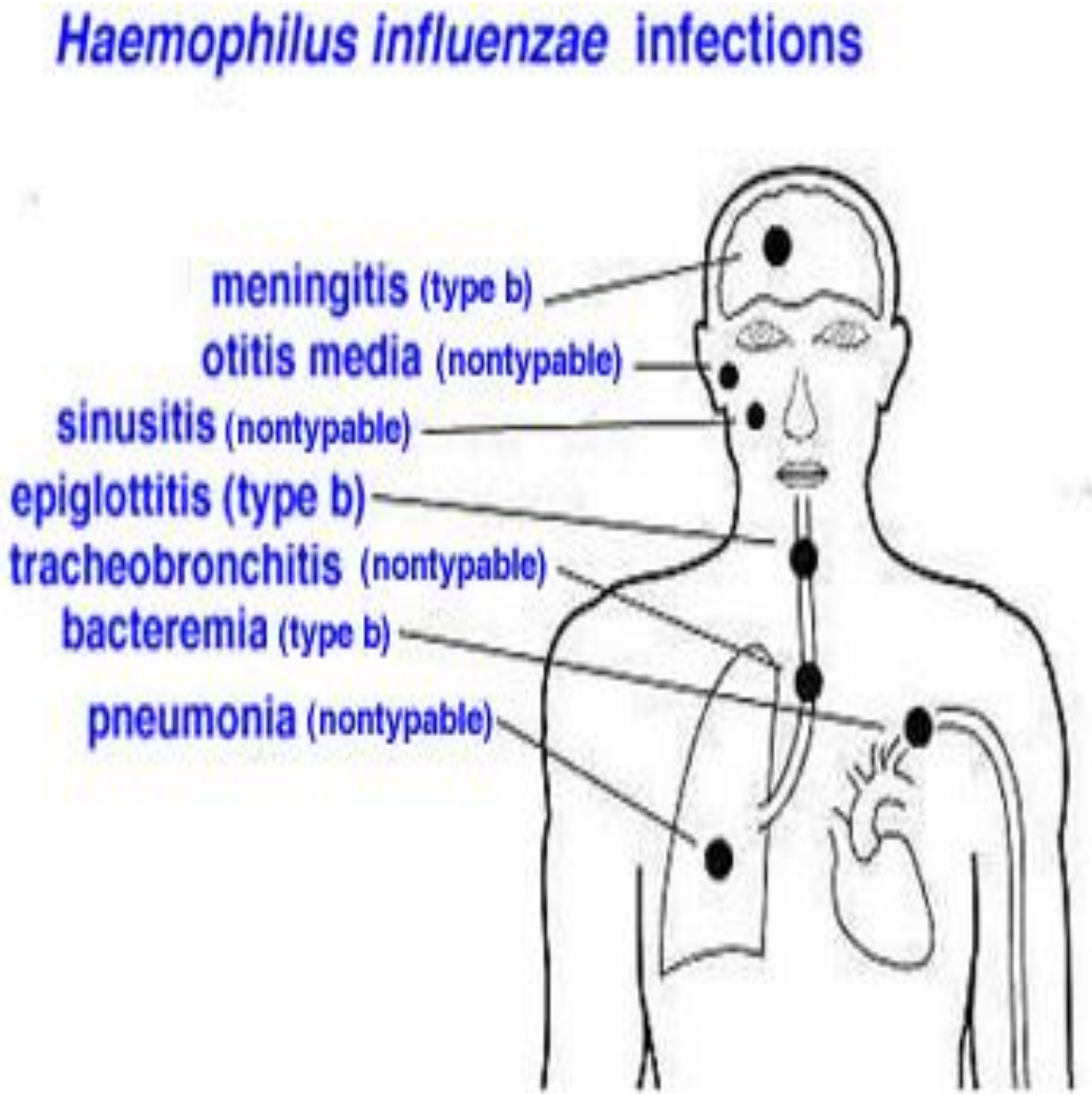
Epiglottitis

Meningitis

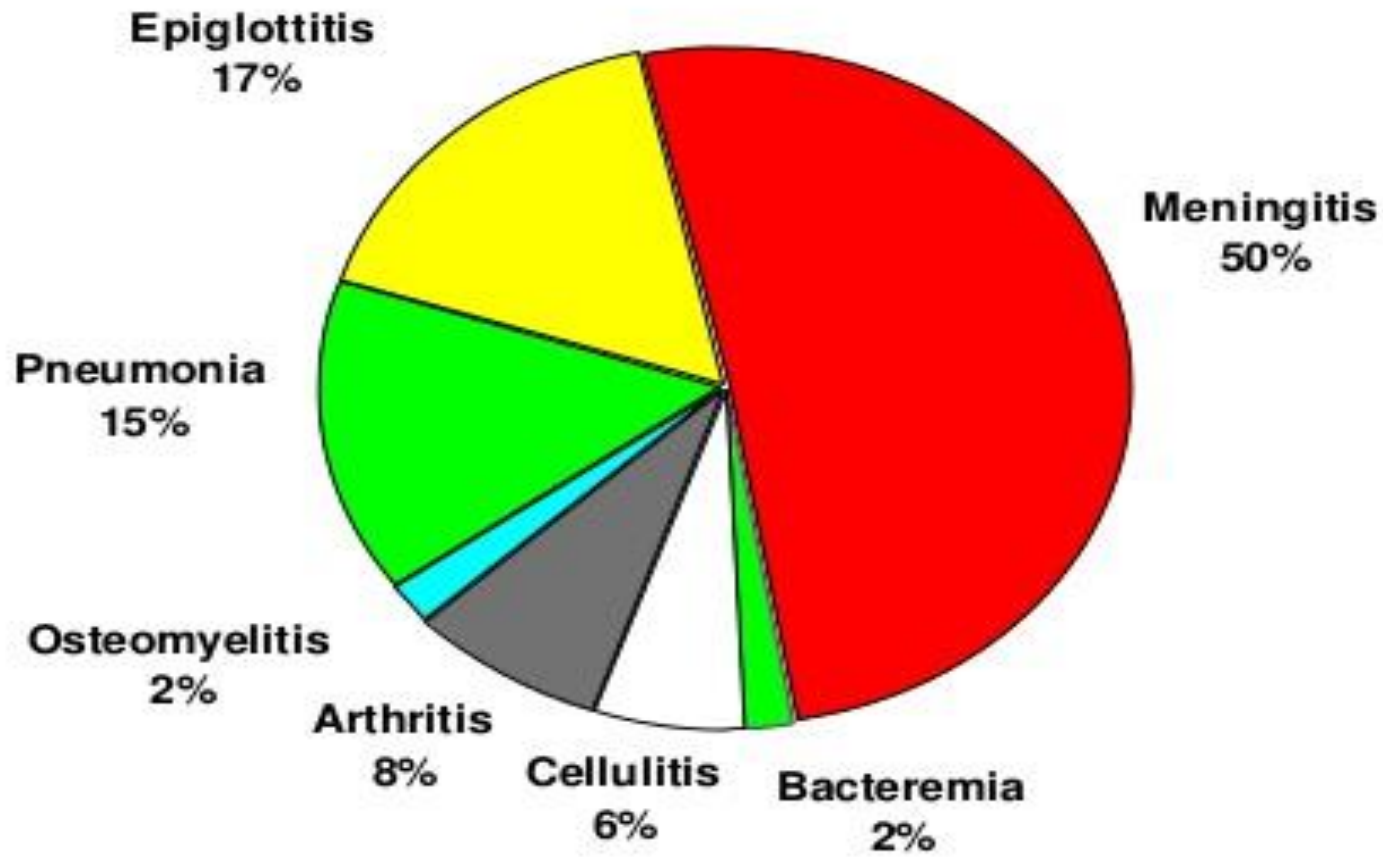
Invasion infection

Secondary infection

- Respiratory tract infections
- Otitis media
- Sinusitis
- Chronic Bronchitis

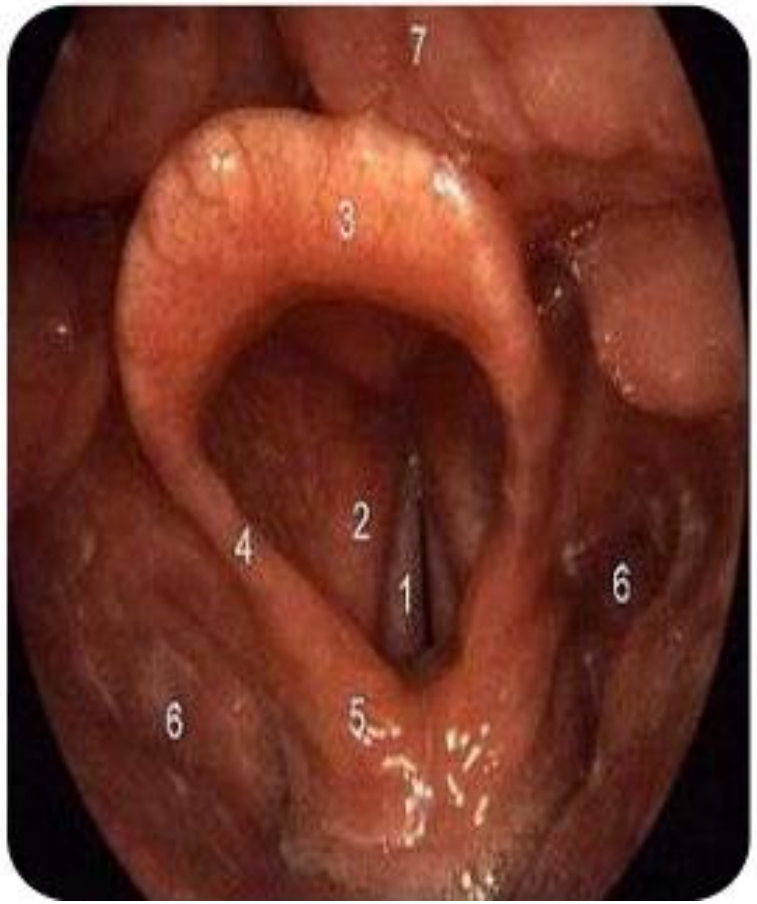


Haemophilus influenzae type b
Clinical Features*



Laryngo epiglottitis

- Causes Epiglottis
- Obstructive Laryngitis
- > 2 years children are vulnerable
- Can be fatal in 2 hours



Laboratory Diagnosis

Sample -: CSF, blood, throat swab, sputum, pus, aspirates from joints, middle ears or sinuses etc

Direct examination

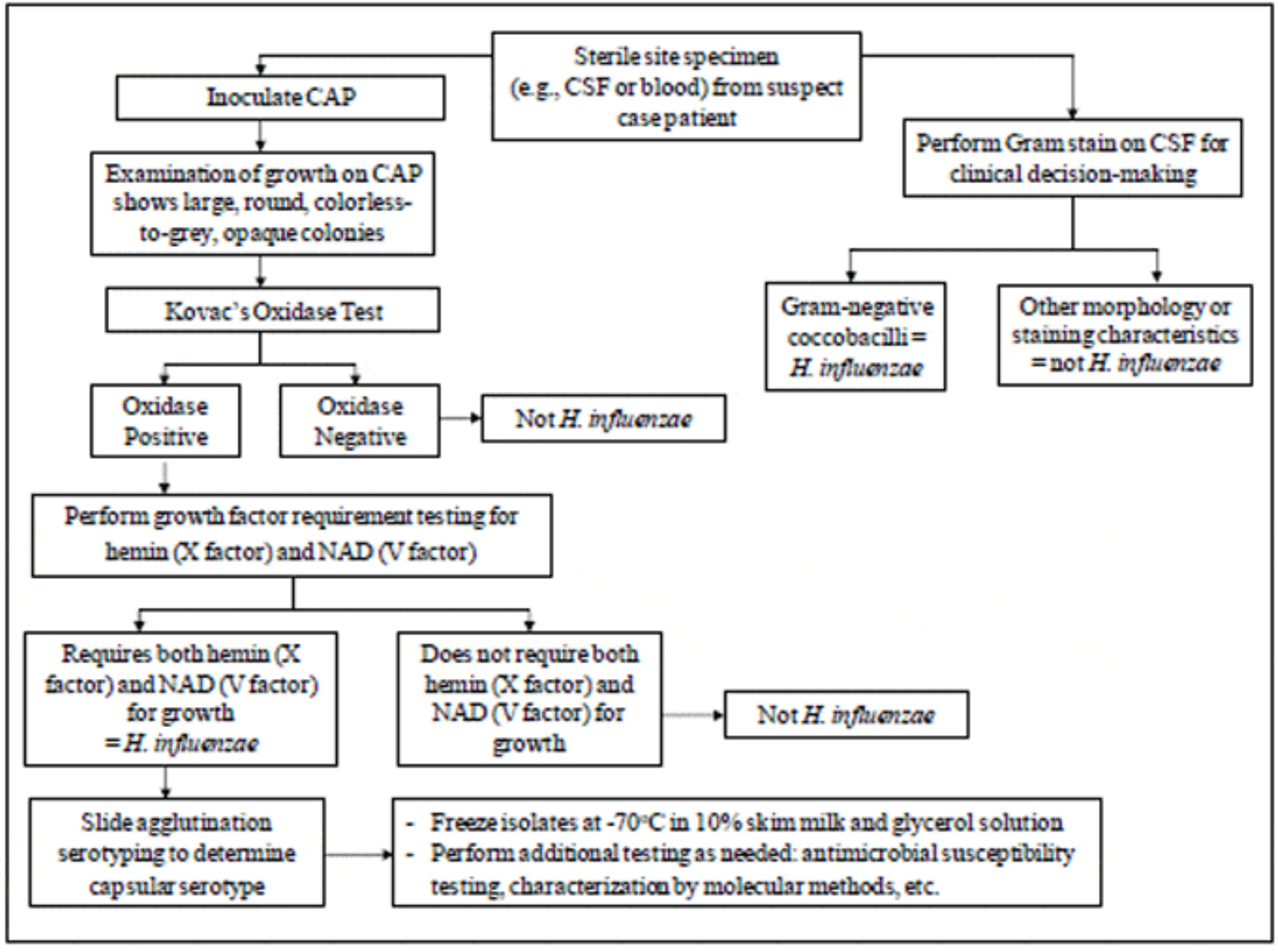
- Gram staining
- Immunoflurescence and quelling reaction

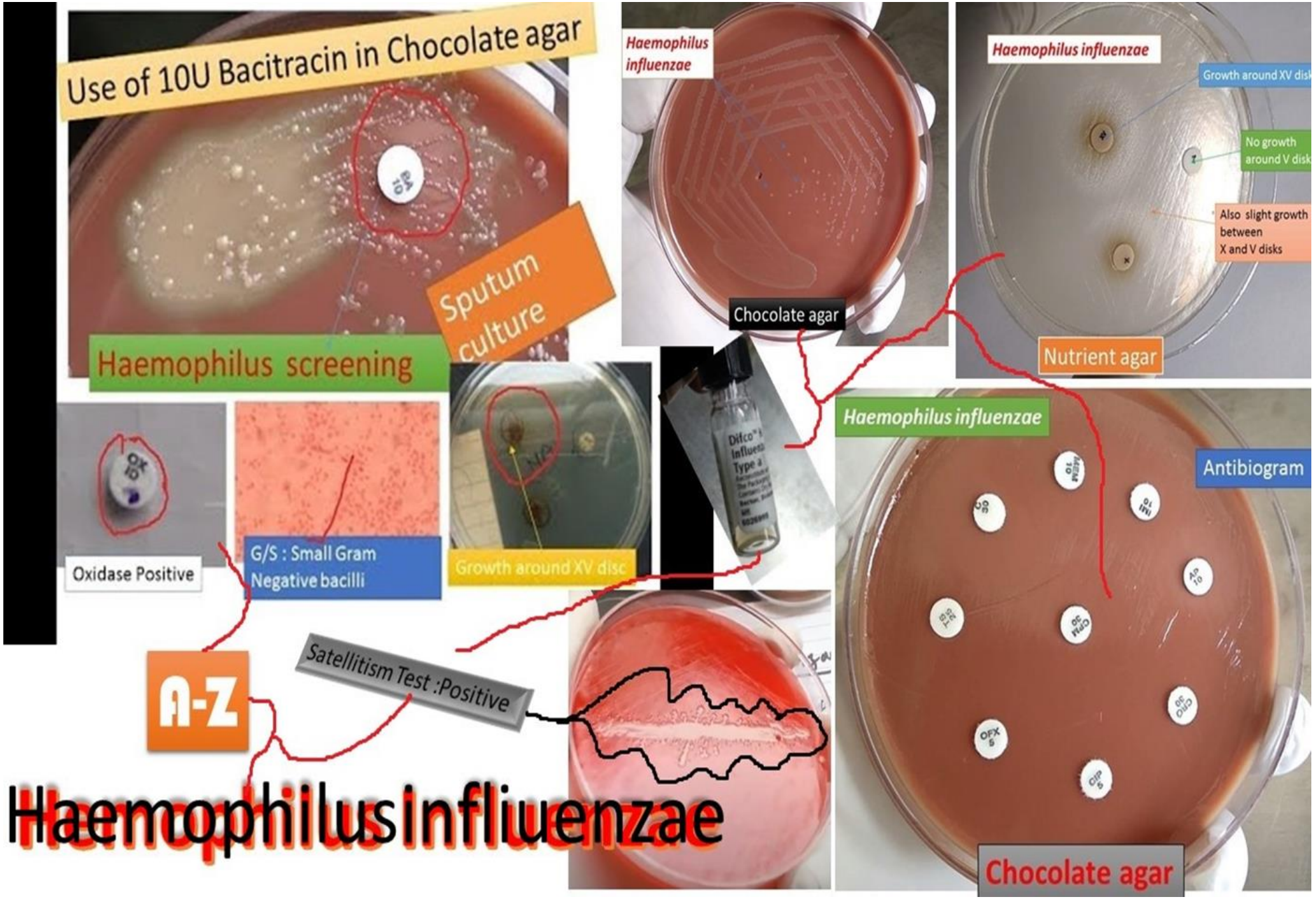
Lab diagnosis

- Type b Capsular antigen detection
  Agglutination of latex particles
  Coagglutation test
  Counterimmunoelectrophoresis (CIE)

Culturing and Isolation

- Can be grown on Blood agar and Chocolate agar
- Need 5 – 10 % carbon dioxide
- A streak of Staphylococcus should be streaked across the plate at 370c
- Opaque colonies appear shows as Satellitisim
- Iridescence Demonstrates on Leviathan medium
- Blood culture and CSF culture


Sterile site specimen (e.g., CSF or blood) from suspect case patient
Inoculate CAP
Examination of growth on CAP shows large, round, colorless-to-grey, opaque colonies
Kovac's Oxidase Test
Oxidase Positive
Oxidase Negative
Not H. influenzae
Perform growth factor requirement testing for hemin (X factor) and NAD (V factor)
Requires both hemin (X factor) and NAD (V factor) for growth = H. influenzae
Does not require both hemin (X factor) and NAD (V factor) for growth
Not H. influenzae
Slide agglutination serotyping to determine capsular serotype
- Freeze isolates at -70°C in 10% skim milk and glycerol solution
- Perform additional testing as needed: antimicrobial susceptibility testing, characterization by molecular methods, etc.
Perform Gram stain on CSF for clinical decision-making
Gram-negative coccobacilli = H. influenzae
Other morphology or staining characteristics = not H. influenzae


Use of 10U Bacitracin in Chocolate agar
Sputum culture
Haemophilus screening
Haemophilus influenzae
Chocolate agar
Haemophilus influenzae
Growth around XV disk
No growth around V disk
Also slight growth between X and V disks
Nutrient agar
Oxidase Positive
G/S : Small Gram Negative bacilli
Growth around XV disc
Haemophilus influenzae
Antibiogram
Satellitism Test :Positive
A-Z
Chocolate agar
Haemophilus Influenzae

Treatment

- Cefotaxime
- Ceftazidime
- Ampicicillin, Contrimixazole
- Plasmid born resistance set in Ampicillin
- Amoxycillin with Clavulanate
- Clarithromycin
- Treatment with an effective 3rd generation cephalosporin, or chloramphenicol plus ampicillin

❑ Ampicillin-resistant strains

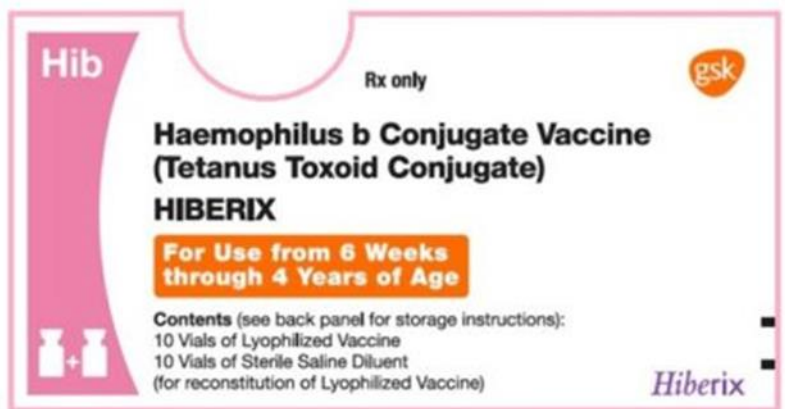
Current Vaccines

- Haemophilus B conjugate vaccine
- Wide spread use of

  H influenza type b vaccine has reduced H influenza type b meningitis in children by 95%

Hib Vaccine

Hiberix

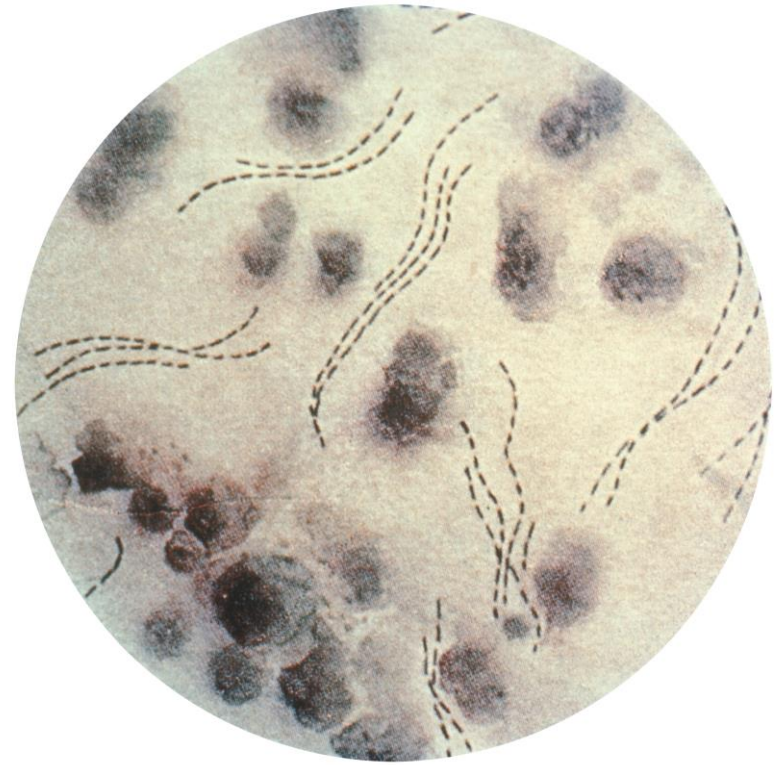


Public Health Aspect of other *Haemophilus* strains

- ***H. ducreyi***
  - **Sexually transmitted disease - chancroid**
- ***H. influenzae* biogroup *aegyptius***
  - **Brazilian Purpuric Fever**
- ***H. aegyptius***
  - **"pink eye" (purulent conjunctivitis)**
- ***H. aphrophilus***
  - **pneumonia**
  - **Infective endocarditis**

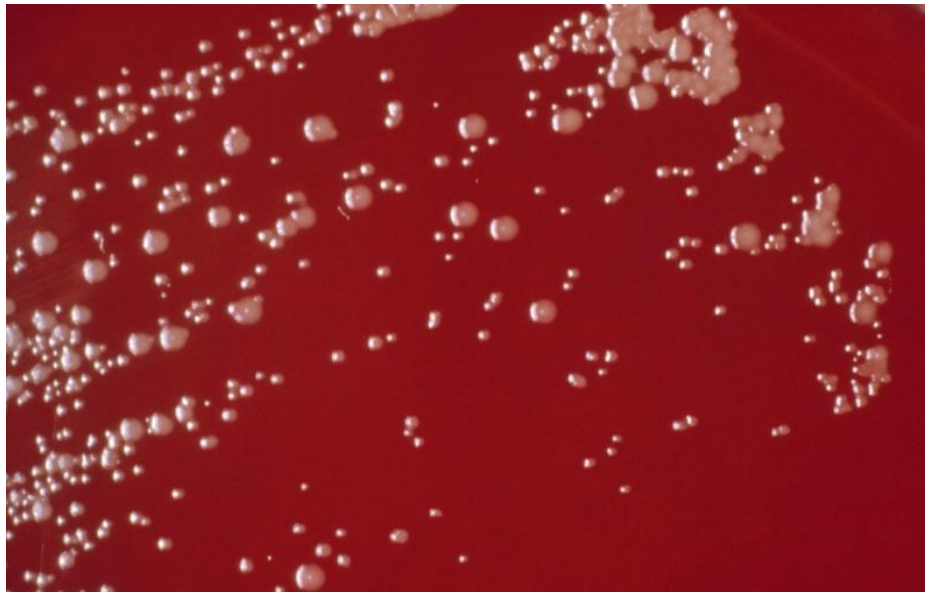
H.ducreyi

- Ducrey 1890
- Short ovoid bacilli
- 1 – 1.5 x 0.6 microns
- End to end pairing in short chains
- Gram –ve appear as Gram +ve
- Bipolar staining
- Bacilli in small groups appear as parallel chains giving school of fish appearance



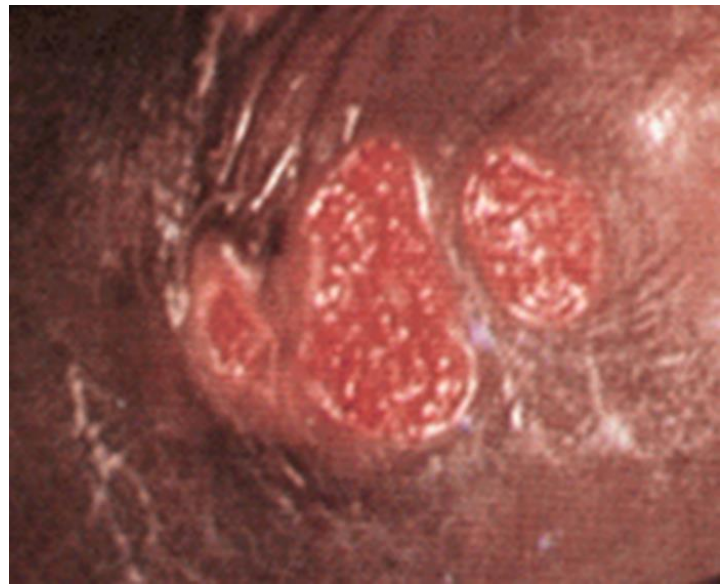
Growth and Culturing

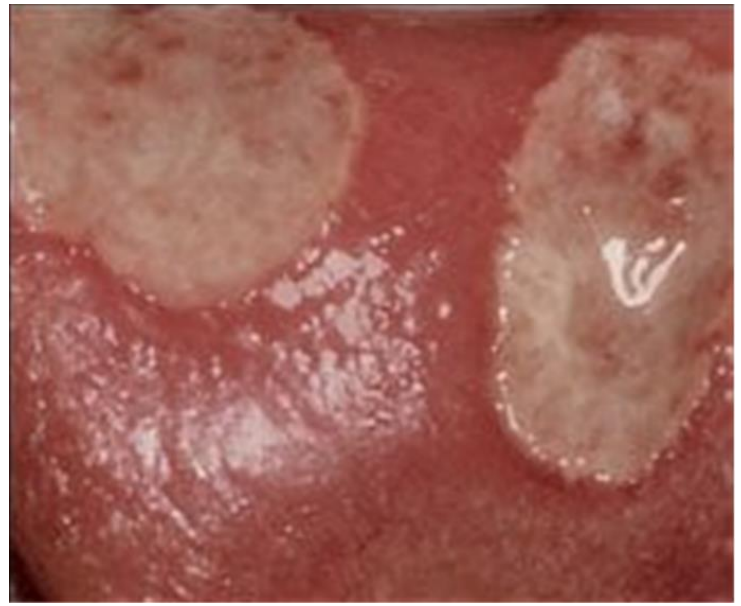
- Grows on Fresh clotted Rabbit blood
- Grows on Chorioallontoic membrane of chick embryo
- Small grey translucent colonies are produced



H.ducreyi

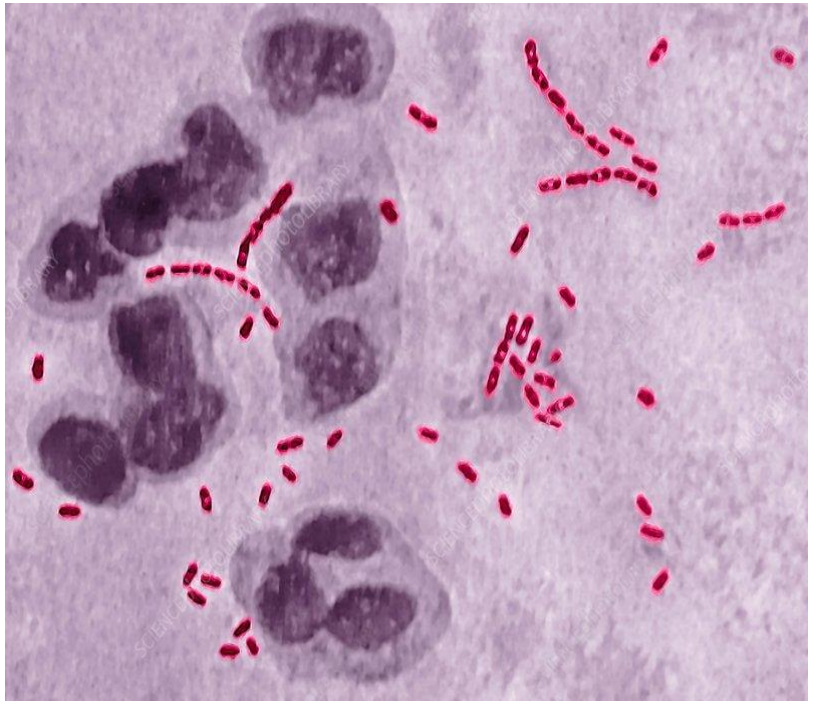
- Seen in genital regions of human
- Can be transmitted by sexually contact – STD
- In men- painful ulcer in genitals, slow healing lymphnodal enlargement, pus formation- CHANCROID- soft based ulcer
- In women – no symptom
- Infection is localized spreading to only in regional lymph nodes





Microbiological diagnostics:

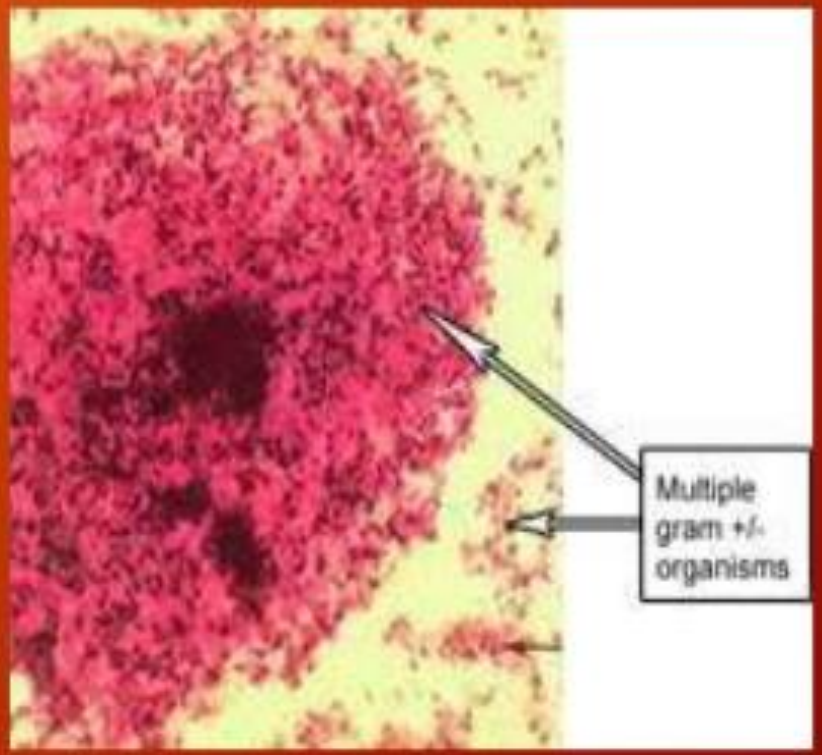
- In smears prepared from chancroid, characterization is based on the **microscopic** detection of small, Gram-negative bacilli with morphology.
- It is possible to obtain a **culture** of the causative agent and identify it by inoculating the pathological material into appropriate nutrient media.
- In some cases, **PCR** is used to identify the causative agent.



Bacterial Vaginosis
(Gardnerella Vaginitis)

Morphology

- Small, Gram negative, on motile
- Pleomorphic rod which shows metachromatic granules
- Presence of Clue cells



Gardnerella Vaginitis

- Gram-variable-staining rod, facultative anaerobic bacteria (actually has a Gram-positive cell wall, but because the cell wall is so thin it can appear either Gram-positive or Gram-negative under the microscope).
- Small (1-1.5 µm diameter) non-spore forming, non-motile coccobacilli.
- Previously classified as Haemophilus vaginalis and afterwards as Corynebacterium vaginalis.

Culturing

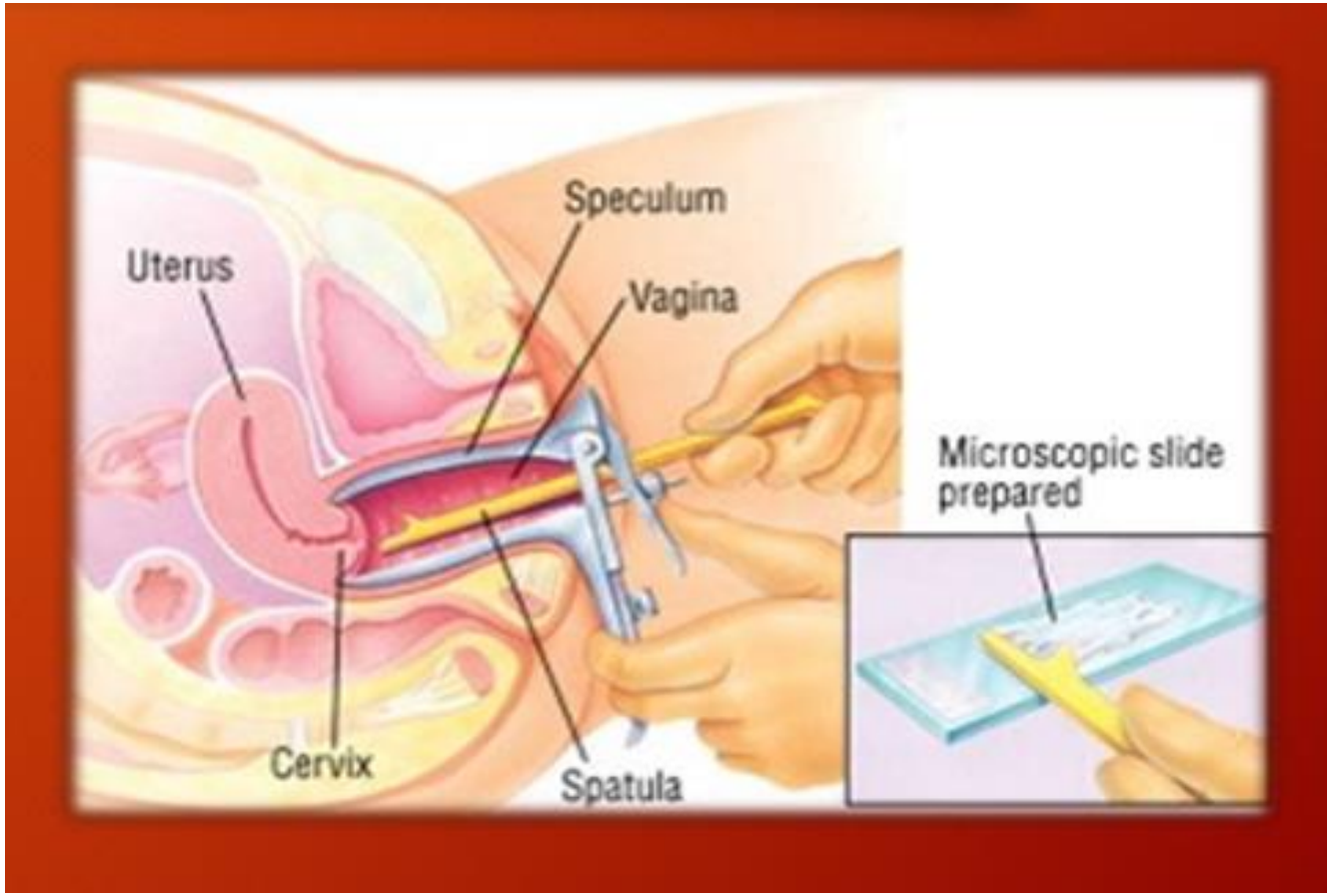
- Grows on Blood and Chocolate Agar
- Hemolytic colonies on Human and Rabbit blood agar,
- Catalase -
- Oxidase -

Symptoms

- Up to 50% of women diagnosed with bacterial vaginosis do not have symptoms. In others, it causes an unpleasant "fishy" vaginal odor and a yellow or white vaginal discharge. For some women, these symptoms are especially bothersome during or after intercourse.

Diagnosis

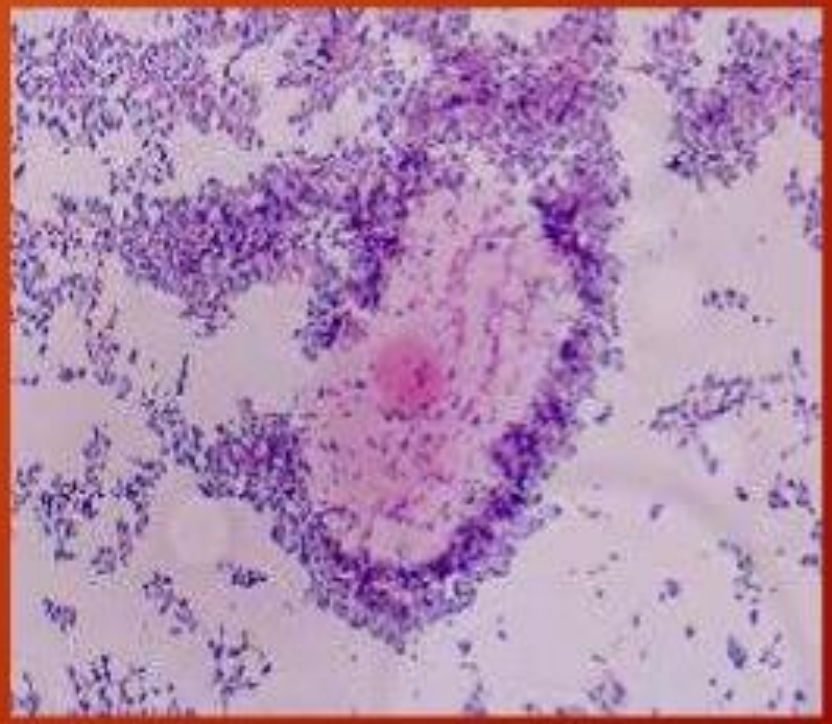
- 1 White, thin, coating on your vaginal walls during the pelvic exam



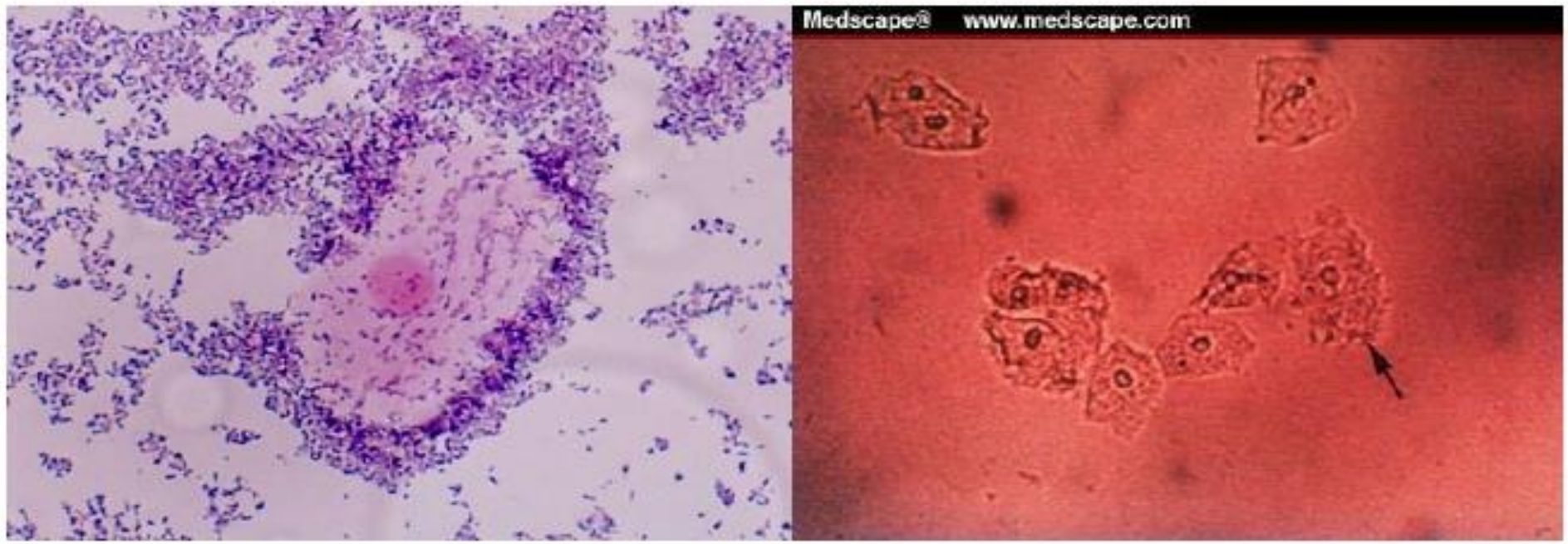
- 2 pH test of vaginal discharge that shows low acidity (pH greater than 4.5)
- 3 Fishy odor when a sample of vaginal discharge is combined with a drop of potassium hydroxide on a glass slide (the "whiff test")

Clue cells

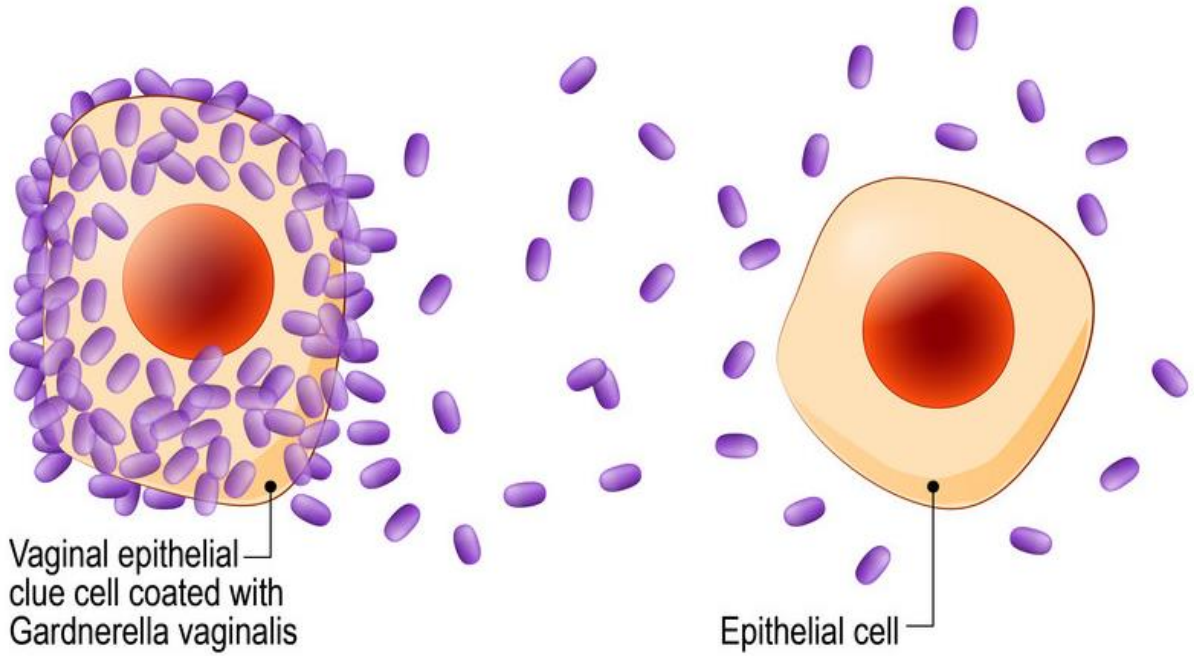
- 4 Clue cells (vaginal skin cells that are coated with bacteria) visible on microscopic exam of vaginal fluid

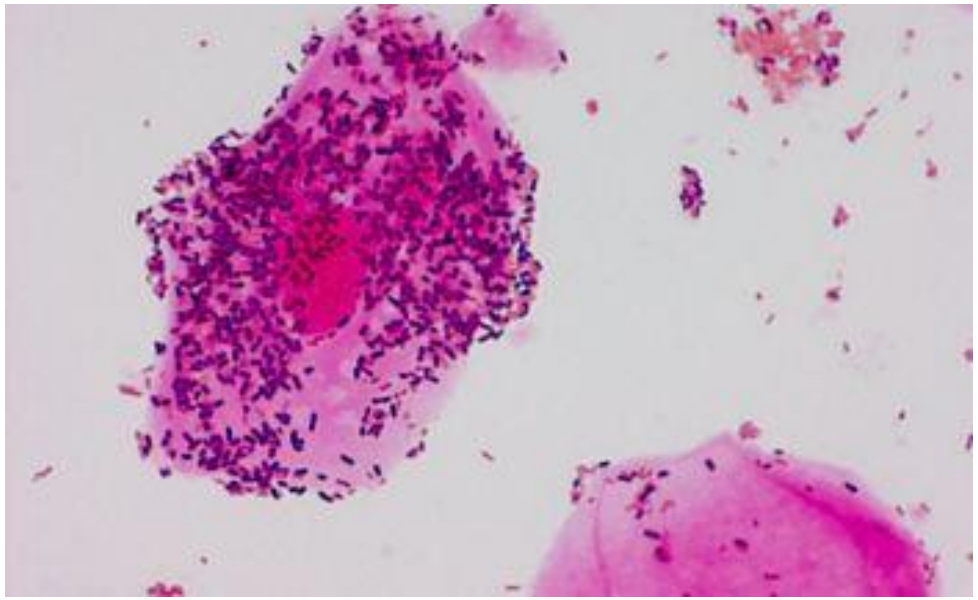


Clue cell



Clue cell on Gram stain and saline wet mount of vaginal discharge (on >20% cells)
Bacteria adhered to epithelial cells; most reliable single indicator


Vaginal epithelial
clue cell coated with
Gardnerella vaginalis
Epithelial cell



Treatment

- Studies show that a seven-day treatment with oral metronidazole or a five-day treatment with metronidazole vaginal gel is equally effective in non-pregnant women. Clindamycin vaginal cream is slightly less effective than either type of metronidazole.

Legionella - Taxonomy

- (Domain): Bacteria
- (Kingdom): Pseudomonadota
- (Class): Gammaproteobacteria
- (Order): Legionellales
- (Family): Legionellaceae
- (Genus): Legionella
- (Species***):*** ***L.pneumophila,*** *L.micdadei*

Legionella pneumophila

Historical Background and Epidemiology

Historical Background

The name legionella originates from a widely publicized outbreak of pneumonia in persons attending an American Legion convention in Philadelphia in 1976.

In a hotel on the occasion of a United States army veterans' meeting (Fraser et al., 1977).

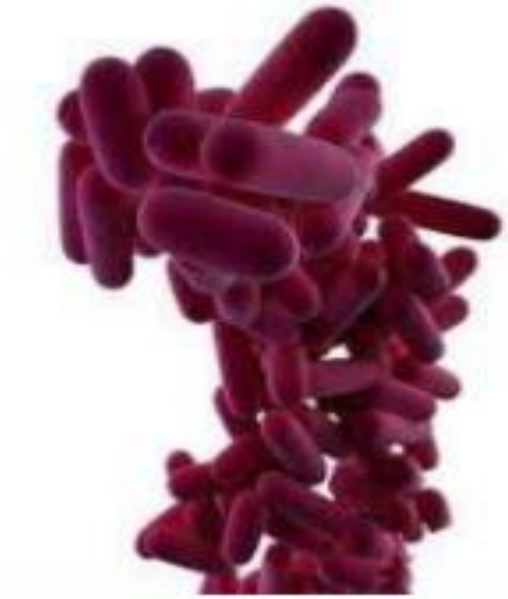
Bacterial Characteristics

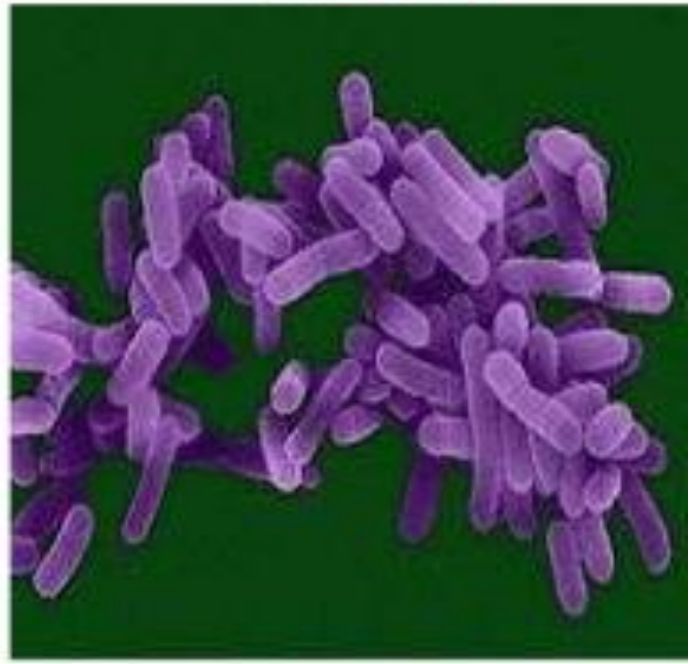
Legionellaceae are facultative intracellular parasites that cause primarily respiratory tract infections.

Legionella are :

- gram–negative
- slender rods
- unencapsulated
- fastidious,
- Aerobic
- catalase-positive
- Most produce gelatinase and ß-lactamase
- 0.5–1 m wide and 2–50 m long.
- poorly stained by Gram's stain

This has been attributed to the presence of the branched chain fatty acids that are a major component of the cell walls.





- Motile by means of one or more *polar* or *subpolar flagella*
- grown on complex media such as *buffered charcoal-yeast extract (BCYE) agar* with ;

  ketoglutarate,

  pH of 6.9,

  temperature 35 °C, and

  90% humidity.
- Legionellae grow slowly;

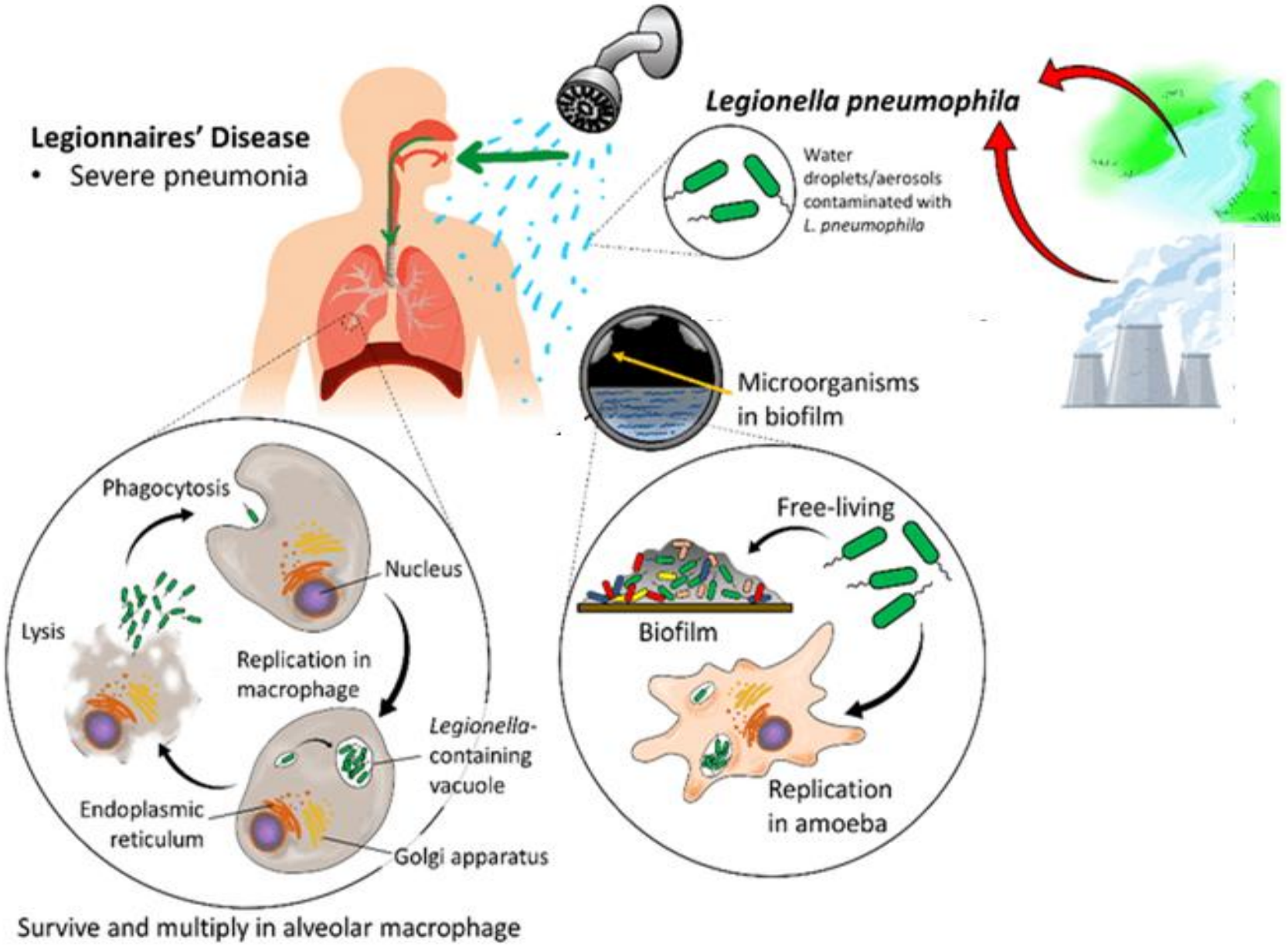
  3 days of incubation with BCYE & ≥2 weeks in blood cultures
- Colonies are round or flat, colorless to iridescent pink or blue

Virulence factors

The Legionellae make:

- proteases,
- phosphatase,
- lipase,
- DNase, and
- Rnase
- A major secretory protein, a ***metalloprotease,*** has hemolytic and cytotoxic activity; however, this protein **has not been shown** to be a required virulence factor.

Source of infection and mode of transmission



Pathogenesis

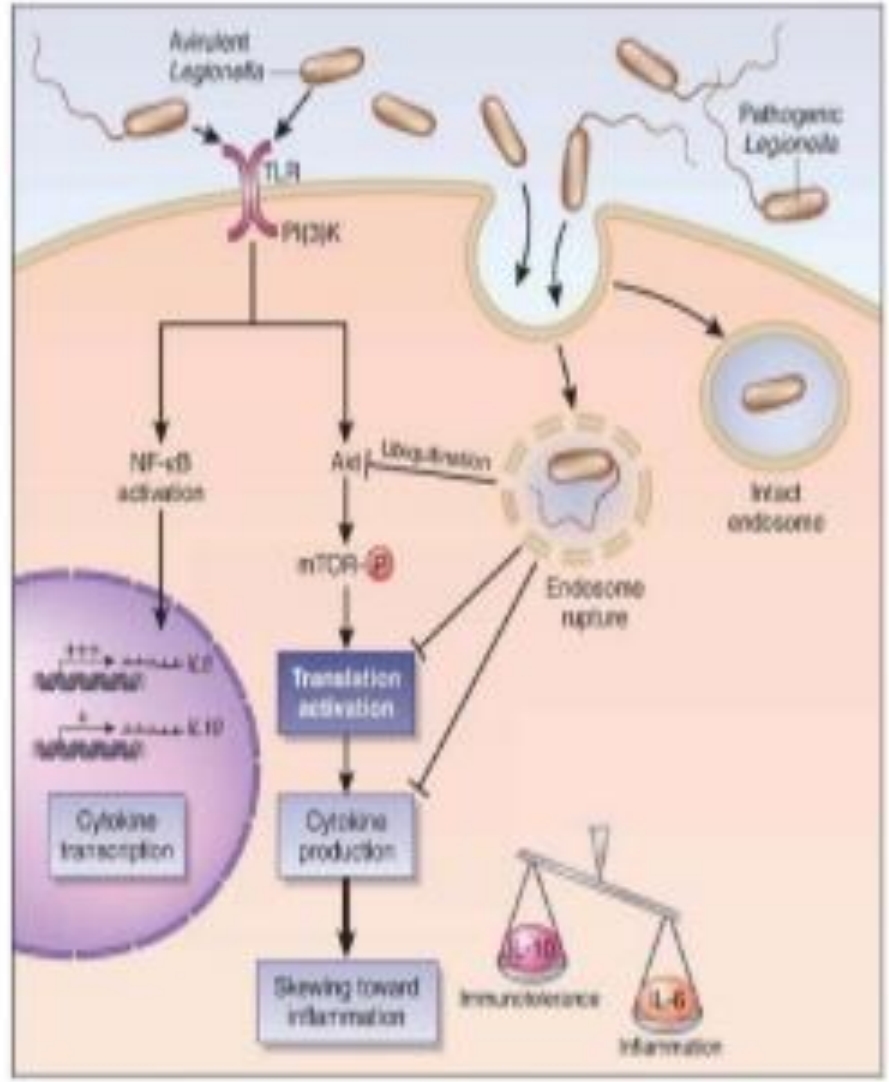
Legionellae are intracellular pathogens of ***macrophages***, by which they are phagocytosed in a process involving ;

1. Both virulent and non-virulent strains are *phagocytosed*
2. Virulent strains can multiply inside the phagocytes and are able to ***inhibit the fusion of phagosomes with lysosomes***

- ❑ non-virulent strains do not multiply

3. The bacteria multiply within the vacuoles until they are numerous,
4. The cells are destroyed, the bacteria are released, and infection of other macrophages then occurs.

(transferrin-iron) is essential for the process of *intracellular growth* of the bacteria, but other factors important to the processes of *growth, cell destruction, and tissue damage* are not well understood.



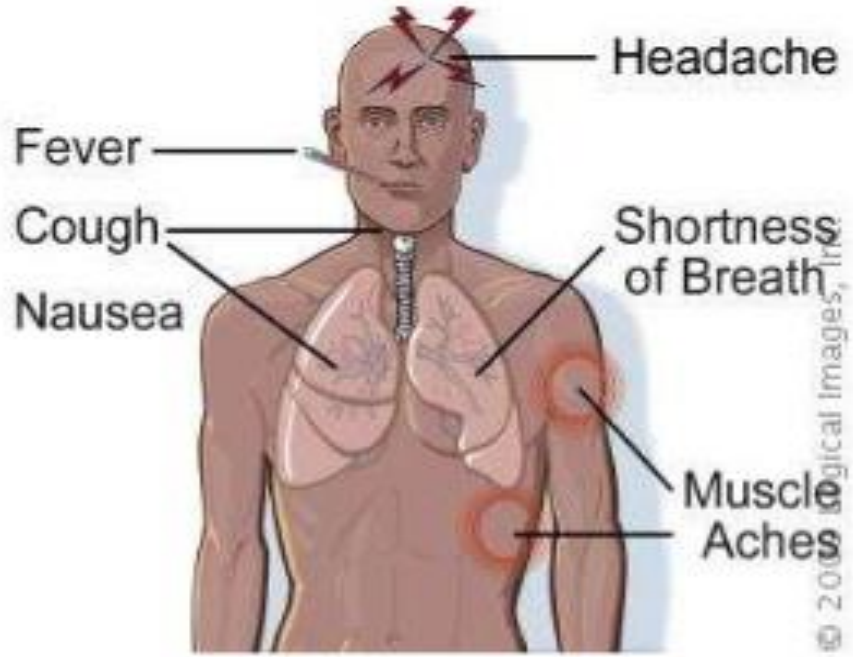
Clinical manifestations

- *L.neumophilia* causes **Legionnaires' disease** can have symptoms like many other forms of pneumonia, so it can be hard to diagnose at first.

Signs of Legionnaires' disease can include:

- Cough
- Shortness of breath
- High fever
- Muscle aches
- Headaches

These symptoms usually begin 2 to 14 days after being exposed to the bacteria.



Potanic fever

- *L .pneumophila* also produces a disease called **"Pontiac fever,"** after the clinical syndrome that occurred in an outbreak in Michigan.

characteristics

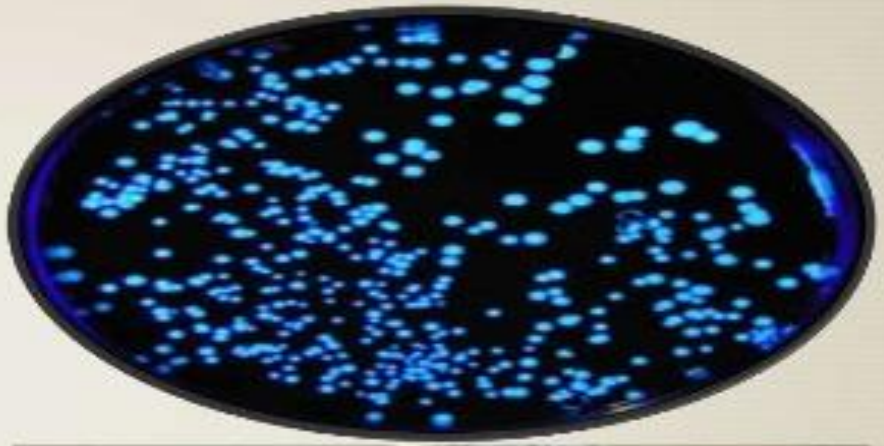
- ❑ fever and chills,
- ❑ myalgia,
- ❑ malaise, and
- ❑ headache ,that develop over *6–12 hours*. Dizziness, photophobia, neck stiffness, and confusion also occur.

- The **symptoms of Pontiac fever** are similar to those of Legionnaires' disease and usually last for 2 to 5 days. Pontiac fever is ***different from Legionnaires' disease*** because the patient **does not** have pneumonia.
- Symptoms go away on their own without treatment.

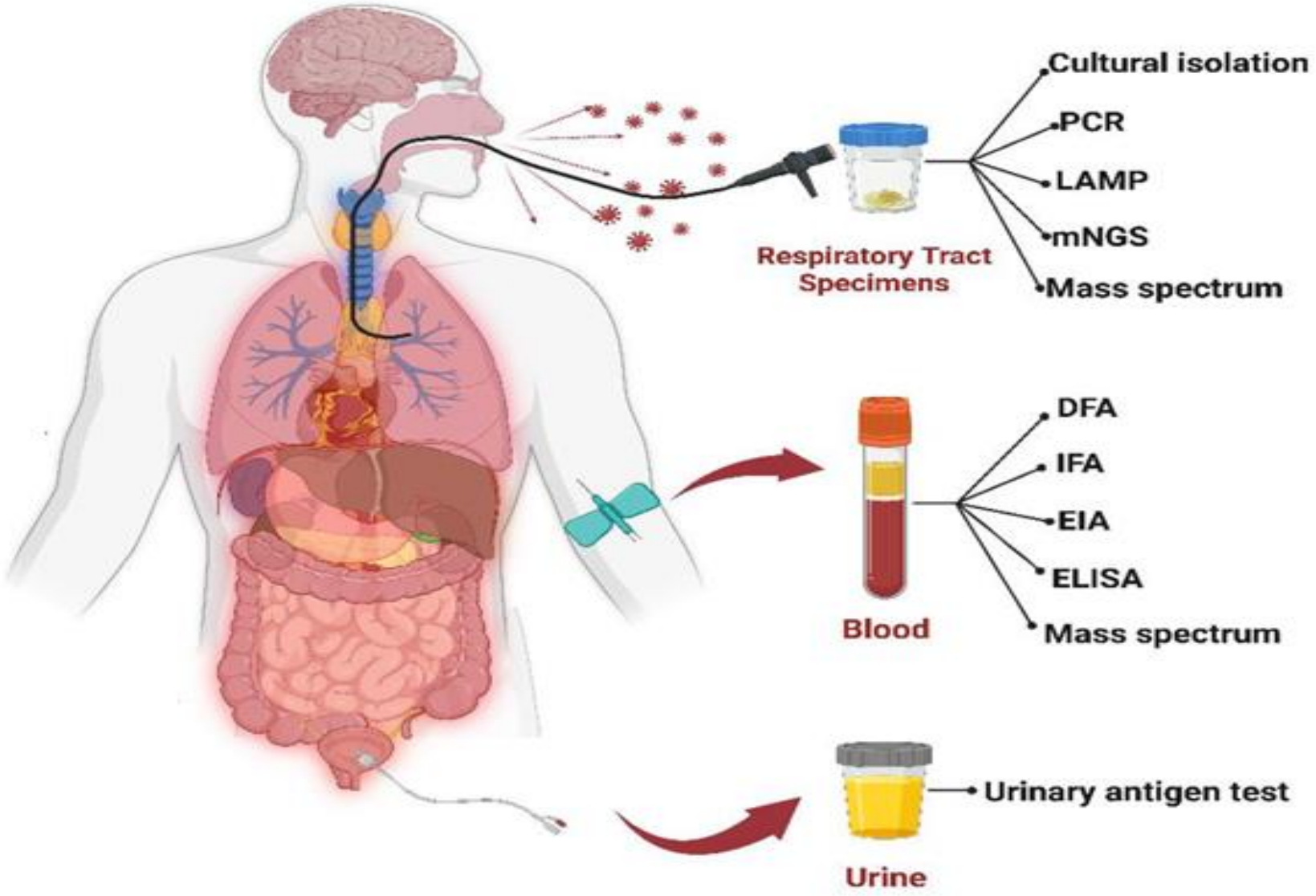
Laboratory Diagnosis

- Specimens
  - Sputum
  - Bronchial aspirate
  - Lung biopsy
  - Florescent methods

Serology ELISA



Microbiological diagnostics



Specimens

The organisms can be recovered from:

- bronchial washings
- pleural fluid
- lung biopsy specimens or
- blood

❖ Isolation of legionella from sputum is more difficult because of the predominance of bacteria of the normal flora. Legionella is rarely recovered from other anatomic sites.

Smears

- Legionellae are ***not*** demonstrable in Gram-stain
- *Direct fluorescent antibody tests*, but the test has low sensitivity compared with culture
- *Silver stains* are sometimes used on tissue specimens.

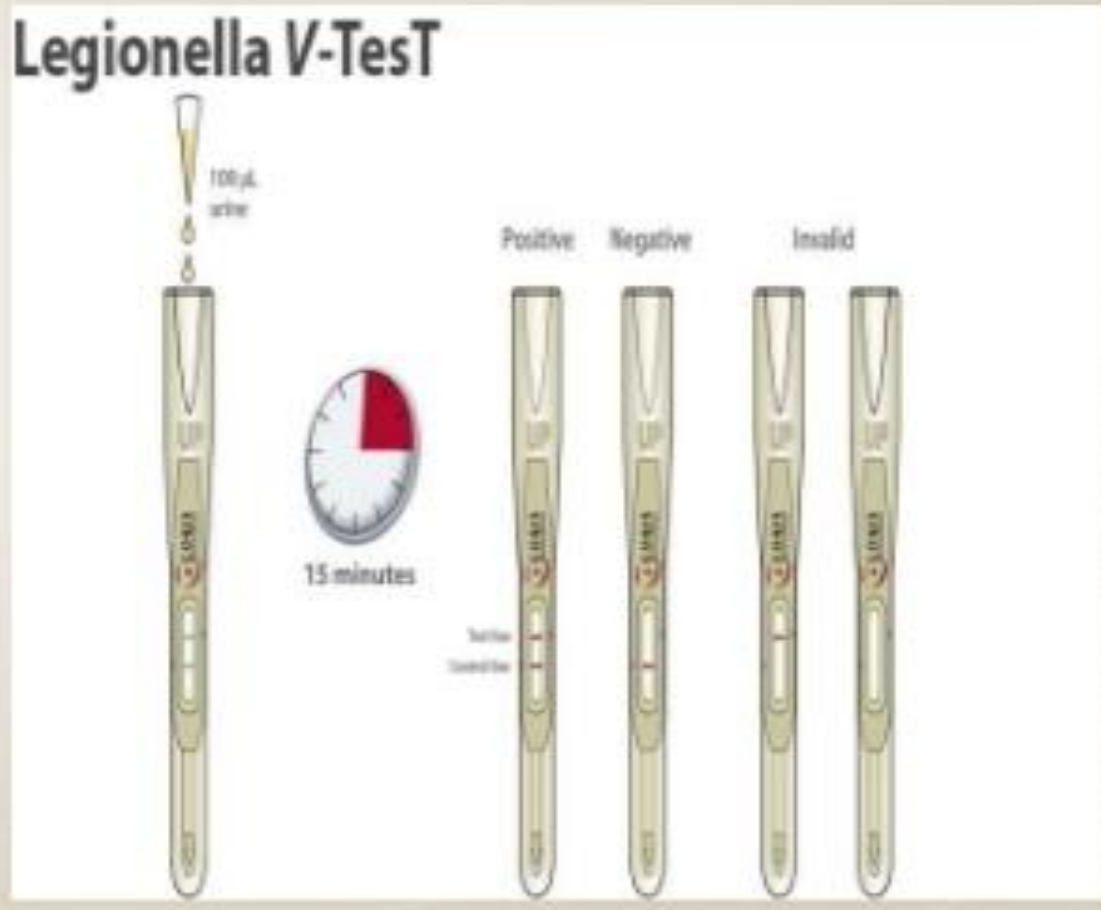
Culture

Specimens are cultured on **BCYE agar** & can be rapidly identified by immunofluorescence staining.

BCYE agar containing antibiotics can be used

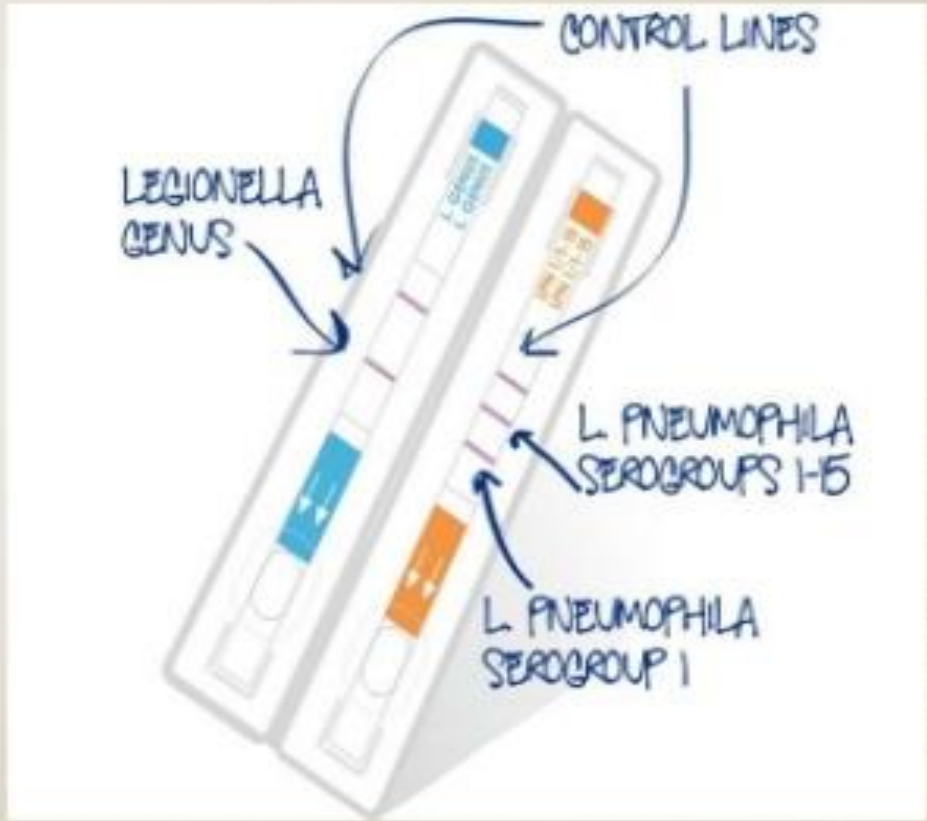
Urine Antigen Test

- The most commonly used laboratory test for diagnosis is the urinary antigen test, which detects a part of the Legionella bacteria in urine (pee). If the patient has pneumonia and the test is positive, then the patient is considered to have Legionnaires' disease.



Blood Specimens
Testing the Serum

- Paired sera (blood specimens) that show a four-fold increase in antibody levels when drawn shortly after illness and several weeks following recovery, can also be used to confirm the diagnosis.



Treatment

* Macrolides
* Ciprofloxacin
* Tetracycline's
* Rifampicin