PASSION ACADEMIC TEAM yu - MEDICINE

Sheet# 2 - MICROBIOLOGY

Lec. Date : 18/2/2020 - THURSDAY

Lec. Title : Heamophilus/Bordetella/ corynebactirium/respiratory viruses

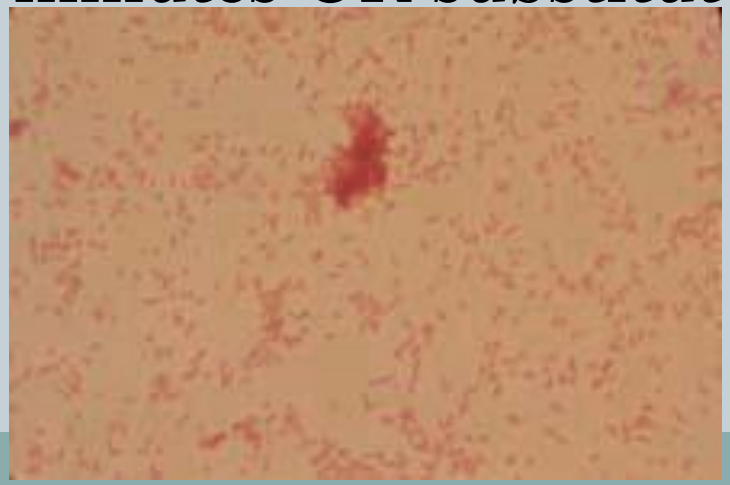
Written By : Ahmad Daas , Abdullah Alsmadi

If you come by any mistake , please kindly report it to shaghafbatch@gmail.com

RESPIRATORY SYSTEM

Haemophilus Species

- Gram Stain Morphology
  - Usually very small pleomorphic gram negative coccobacillus or rod
  - May be able to observe a halo around the organism
  - Gram stain can be enhanced by extending time for safranin to 2 minutes OR substitute carbolfuschin for safranin



Haemophilus Species

- Colony Morphology
  - No growth on BAP or MAC
  - On CA:
    - semi-opaque, gray-white, convex, mucoid.

Haemophilus Species: Identification

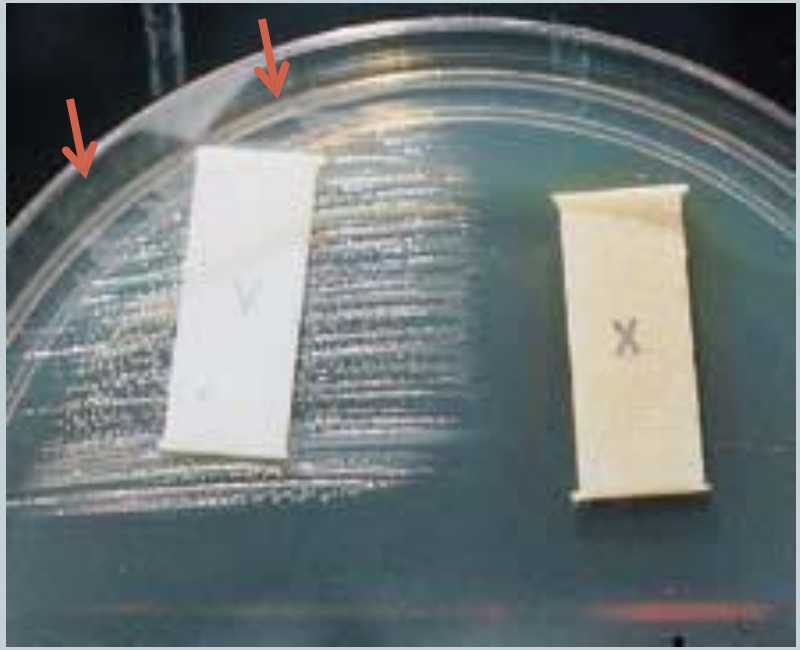
- Gram stain
  - Gram negative cocco-baccillus
- Catalase +
- Oxidase +
- X and V factor strips or disks

  - We use X factor and V factor to Differentiate between different species of Haemophilus
  - some need V factor and some need X factor and some need both of them for example:

Haemophilus influenza needs both of them (X and V factors)

Haemophilus Species: Identification

- parainfluenzae Needs only V factor
- There is Heavy Growth near to V factor but there is no Growth near to X factor



This organism would be identified as *H. parainfluenzae* because it is using V factor only.

Transmission

- H. influenzae, including Hib, are spread person-to-person through respiratory droplets.

- Most of the time asymptomatic

All The respiratory pathogen transmitted by aerosol

Epidemiology

The target population of H. influenza is

1)Children under 5
2)Elders older than 65
3)People live in crowded areas

- H. influenzae, including Hib, disease occurs mostly in babies and children younger than five years old.

- Adults 65 years or older, American Indians, and Alaska Natives are also at increased risk for getting sick with invasive H. influenzae disease.

Q) Is it related to their Weak immunity??

A) Yes , actually its related to 2 factor :

1)Immunity 2) personal Hygiene 3) the Distance between people

- People with certain medical conditions are also at increased risk for developing H. influenzae disease. Those medical conditions include:

- Sickle cell disease
- Asplenia (no spleen)
- HIV (human immunodeficiency virus) infection
- Antibody and complement deficiency syndromes
- Receipt of chemotherapy or radiation therapy for malignant neoplasms
- Receipt of hematopoietic stem cell

Not certain but they did statistical research and found that people with sickle cell disease have increased risk for developing H. influenzae

Treatment

The drug of choice depend on the case itself for example:
1)If it is clinical case epiglottitis Then : Cefotaxime is the drug of choice
2)Secondary infections : Augmentin is drug of choice

Antimicrobial Susceptibility Testing and Therapy

1. Cefotaxime or Ceftriaxone are drugs of choice

2. Less serious Respiratory Infections e.g. OM, sinusitis & acute bronchitis: Oral antibiotics e.g. amoxicillin, co-amoxiclav (Augmentin) & clarithromycin(Macrolide).

Prevention

Vaccine

The good thing that H. influenza has a Vaccine called Hib vaccine
The capsule is used to make vaccine (toxoid)

- Routine vaccination with protein-polysaccharide conjugated vaccine (Hib)
- Significant reduction of serious, life-threatening infections in children
- Recommended starting at 2 months of age

(Prophylaxis :Rifampicin

Oraly, (20mg/kg, up to 600 mg max.), once daily, for 4 days to eradicate H. flu carriage & prevents infection in household & nursery contacts.

In Winter OR when Child in nursery developed H. influenza we give children prophylactic treatment .

Bordetella

Dr. Waleed Al Momani
Associate professor
Medical microbiology
Faculty of medicine
Yarmouk uninersity

It a Gram negative bacillus
Is very short bacillus

Bordetella sp.

Organism	Reservoir	Transmission
B. pertussis (human pathogen)	Not normal flora; only present during infection	Person-to-person; airborne transmission via cough
B. parapertussis (human pathogen)	Not normal flora; only present during infection	Person-to-person; airborne transmission via cough
B. bronchiseptica	Normal flora of animal upper resp. tract (dogs, cats, rabbits) Pets like cats & Dogs & pigs	Exposure to contaminated droplets following close contact with animals

Clinical characteristics

B. pertussis and *B. parapertussis*

Both Cause Whooping cough
But parapertussis cause a milder disease

-Cause URT infections in humans with almost identical symptoms, epidemiology and therapeutic management

- Pertussis (whooping cough)

B. bronchiseptica

Transmitted through close contact (aerosol) with pets that have *B. bronchiseptica as normal flora of that pet and cause respiratory tract infection*

- Opportunistic infection in compromised patients with history of close animal

Clinical characteristics

Epidemiology

Whooping cough is age related disease
symptom Decrease with aging (this theory work until 65 and it depends on immunity)

- Pertussis primarily caused by *B. pertussis*, rarely by *B. parapertussis*; former cause more severe disease

- higher infection rates and increased duration of symptoms

- Adults and adolescents can serve as reservoirs and transmit to unvaccinated or vaccinated with waning immunity

Clinical characteristics

Pathogenesis

Multiple virulence factors with various functions

1. Adhesion (pilli)

2. Toxicity

Has multiple toxin (5-6 toxins):
1)pertussis toxin (the most important one and the main cause of most of the symptoms of whooping cough like cough ,sneezing, fever ,hypoglycaemia , hypotension)
2)adenylate cyclase : the toxin stop transformation of amp to atp , so it cause accumulation of amp and cause Vomiting in upper respiratory tract (in intestine it cause diarrhea) 'vomiting and diarrhea both cause dehydration.'

Pertussis toxin, adenylate cyclase, Tracheal cytotoxin

Outer membrane inhibits host lysozyme

Asphyxia: اختناق
Dyspnea:ضيق التنفس

Clinical characteristics

Stages of whooping cough

Spectrum of disease

Catarrhal	(Incubation period)	Mild cold	(Mild signs and symptom)	Several weeks
Paroxysmal	(Acute phase of the disease)	Severe coughing "Whooping"	(Dyspnea , Asphyxia , respiratory distress, sneeze)	1 to 4 weeks
Convalescent		↓ Symptoms		Months

Whooping cough has long duration of illness (1-4 months) if its not vaccinated (other respiratory tract infection last for 2 week or less)

Symptoms in adults tend to be milder and are misdiagnosed as bronchitis; also tend to be mixed with other pathogens

Laboratory Diagnosis

Specimen collection

- Nasopharyngeal wash or swab (Calcium alginate or dacron on a flexible wire shaft)
- Swabs should be immediately inoculated onto media and direct smears made at the bedside
- Swabs not directly inoculated should be placed in transport if time to lab is extended

We should move the swab Directly to the culture (on spot (bed side))
If we could not do that ,Then we should use transport medium

Laboratory Diagnosis

Direct detection

DFA: Direct Florescent antibody (serology Technique) (Poor technique)

- DFA of smear using polyclonal Abs against *B. pertussis* and *B. parapertussis*

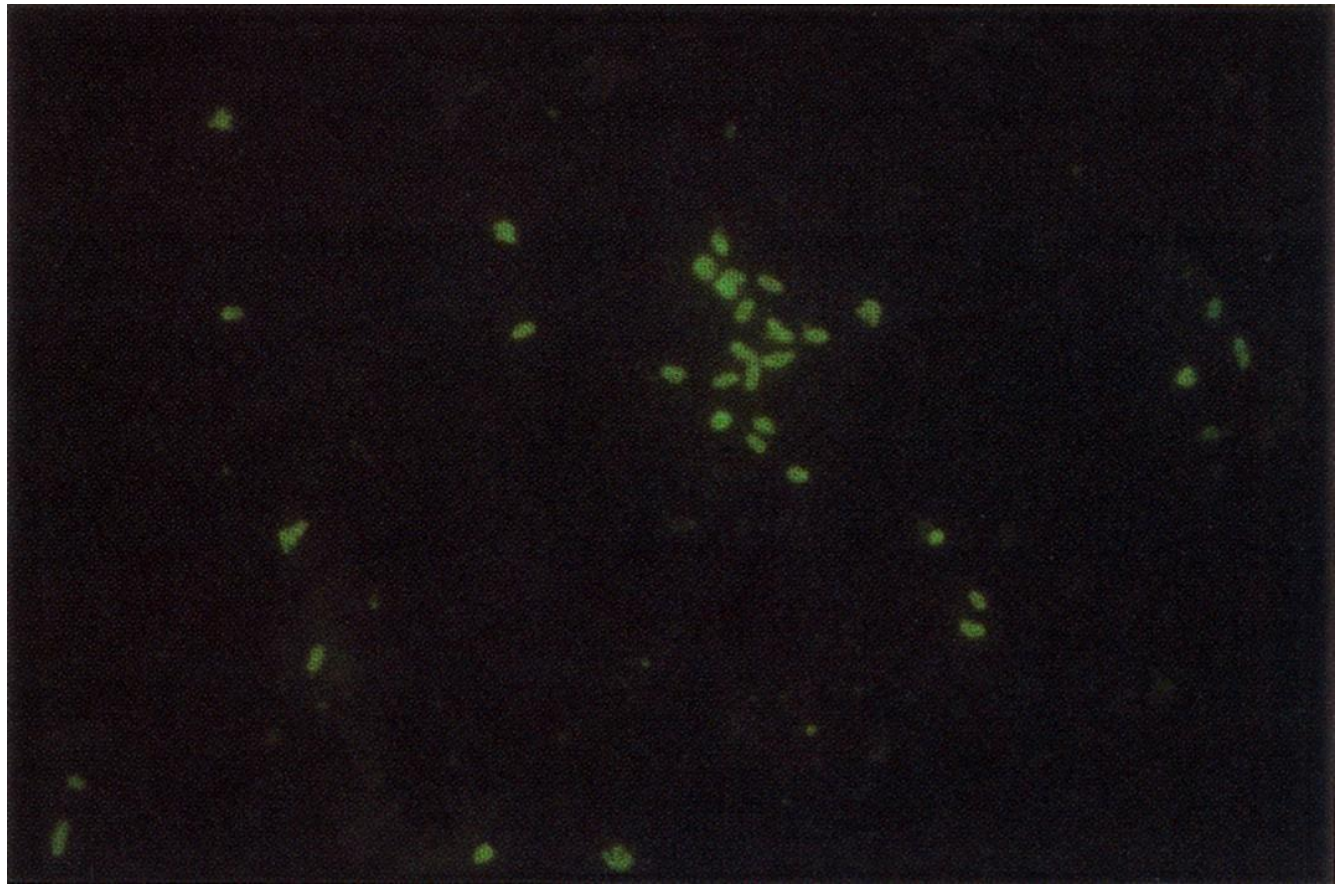
- Sensitivity is limited (50 – 70% at best), so should always be used in conjunction with culture

- PCR methods are increasing in use and are replacing culture as gold standard
  - specificity has been an issue

Don't give us the chance to do Antimicrobial susceptibility test

We usually use both of the tests Culture [redacted] and molecular (PCR) technique as a conformality test

DFA for *Bordetella*



Laboratory Diagnosis

Culture

- Gold standard

- Selective media required
- Bordet-Gengou (only Bordetella pertussis Grow on these media)
- Potato infusion agar with glycerol and sheep blood

- Regan-Lowe
  - Charcoal agar with 10% horse blood
    Cephalexin

Laboratory Diagnosis

Culture

Is slow Growth bacteria (need 10-12 days) to grow other bacteria usually need 12-24 hour

Incubation condition

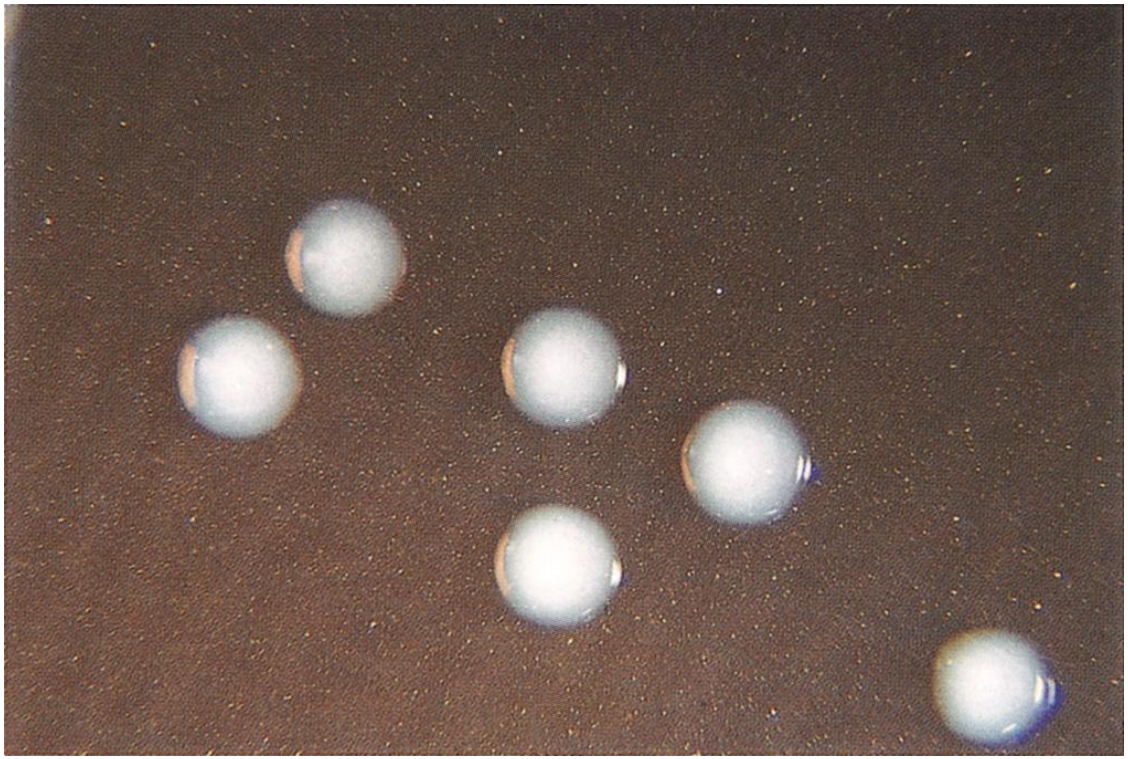
Incubation Time

- 35 – 37°C, 5 – 10% CO_2, hold for 10 – 12 days
  - most isolates are detected in 3 – 5 days

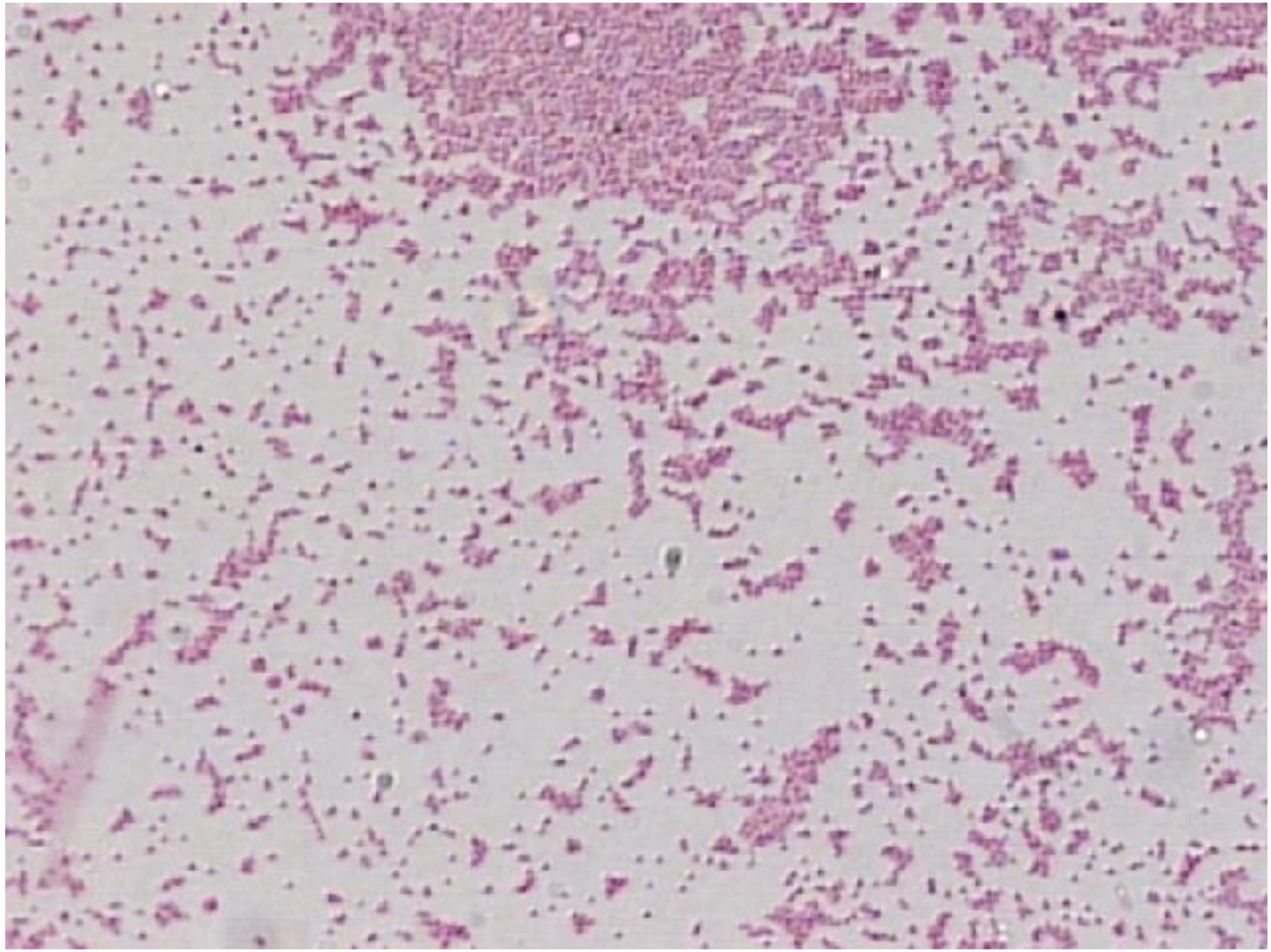
- Colonies are small, shiny; resemble mercury drops

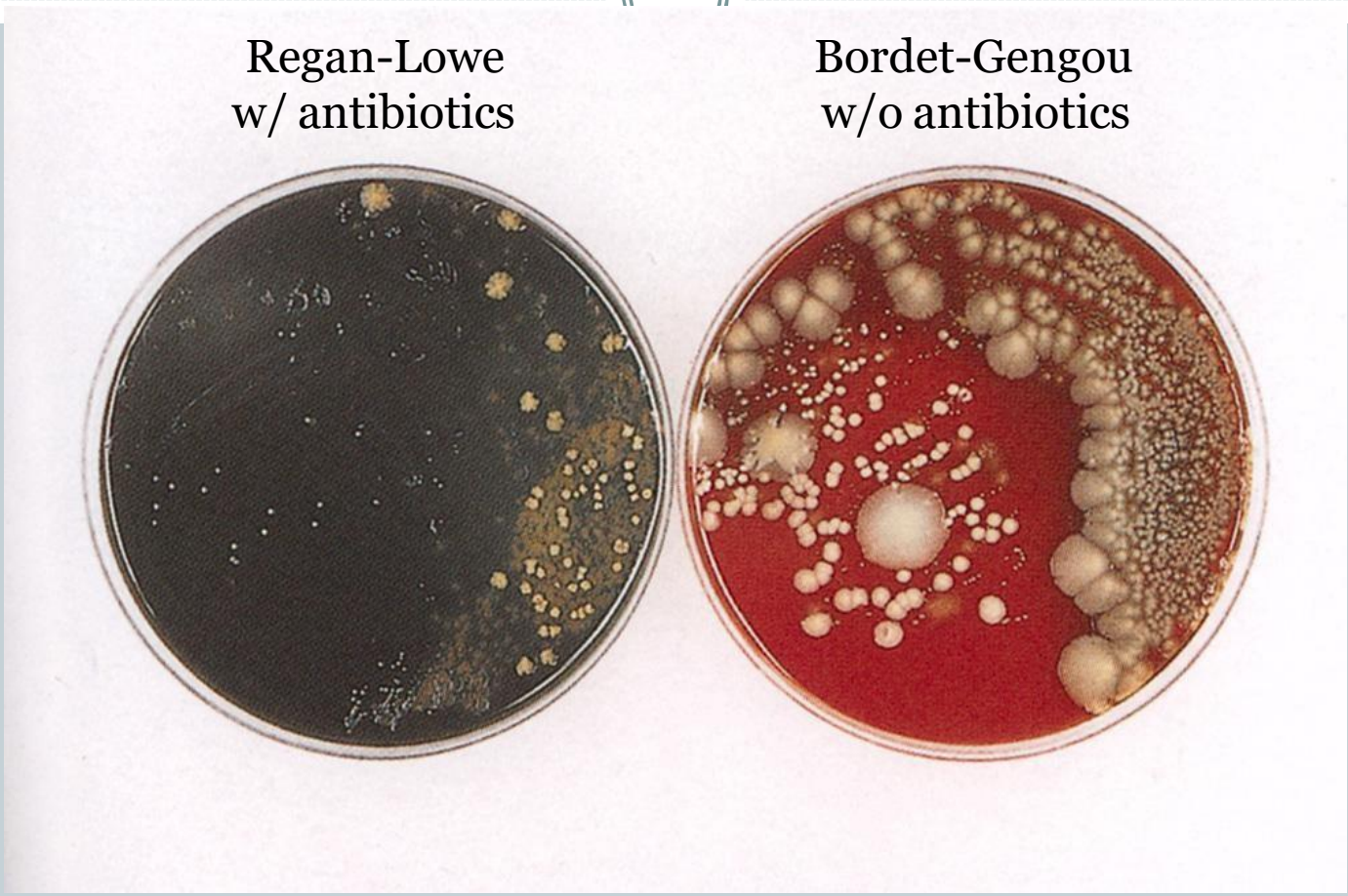
- Gram stain shows small, faintly staining gram negative coccobacilli
  - confirm identity with DFA reagents
  - can distinguish between *B. pertussis* and *B. parapertussis*

B. pertussis on Regan-Lowe agar



Gram stain of *B. pertussis*




Regan-Lowe
w/ antibiotics
Bordet-Gengou
w/o antibiotics

Treatment

Antimicrobial Susceptibility Testing and Therapy

- Not routinely performed because Erythromycin and Azithromycin are active and remain drugs of choice

And Clarithromycin

Now We found a lot of people that have Bordetella That is resistant to Erythromycin because that people take antibiotic
in wrong way like treating virials infection
so bacteria develop Resistance against These antibiotics
Or sometimes they use strong antibiotic to treat simple infections (Start with last step)

Prevention

There is a good vaccine to prevent Bordetella

Vaccine

- Whole-cell vaccines have been used historically
  - adverse reactions and waning immunity

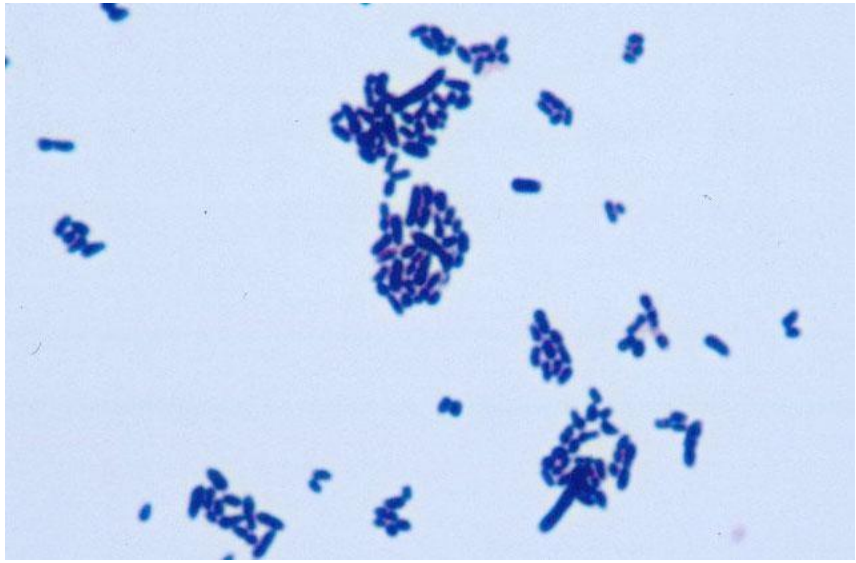
- Acellular vaccines have been developed and include booster doses for older children and adults

Corynebacterium

Corynebacterium Diphtheriae is an acid fast bacteria like M.Tuberculosis , but mycolic acid amount inside them is low so they are lite acid fast bacteria

Corynebacterium

- Mycolic acids: Present in cell wall
- Metachromatic granules can be observed in rods stained with methylene blue , these granules are used for storage of phosphate and we can diagnose corynebacterium by them under microscope
- Metabolism: Fermentative
- “Club”-shaped, non-motile, catalase positive
- Most species grow aerobically on most media
- Lipophilic strains require lipids for good growth
- Anoter feature is “chinese letter arrangement” under microscope when we smear and stain it



C. diphtheriae

Pathogenesis and Immunity

- C. diphtheriae occurs in the respiratory tract. It is spread by droplets or by direct contact.
- Portal of entry: respiratory tract.

- Diphtheria toxin is an A-B toxin expressed from a temperate phage (b-phage) in the presence of low iron concentrations.

- It’s toxin is sub-unite toxin which is very harmful type
- It’s toxin first part has the ability to attach to tissue and the other part will internalize inside the cell

- معلومة خارجية :

- AB toxins are two-component protein complexes secreted by a number of pathogenic bacteria and exert their toxic effects by affecting intracellular processes

Clinical Diseases

Respiratory diphtheria

Starts as any other respiratory infection: coughing, sneezing, fever, …etc

- Incubation period: 2-6 days.
- Inflammation begins in the respiratory tract, causing sore throat, exudative pharyngitis that develops into white pseudomembrane covering the soft palate preventing air passage, and low grade fever.

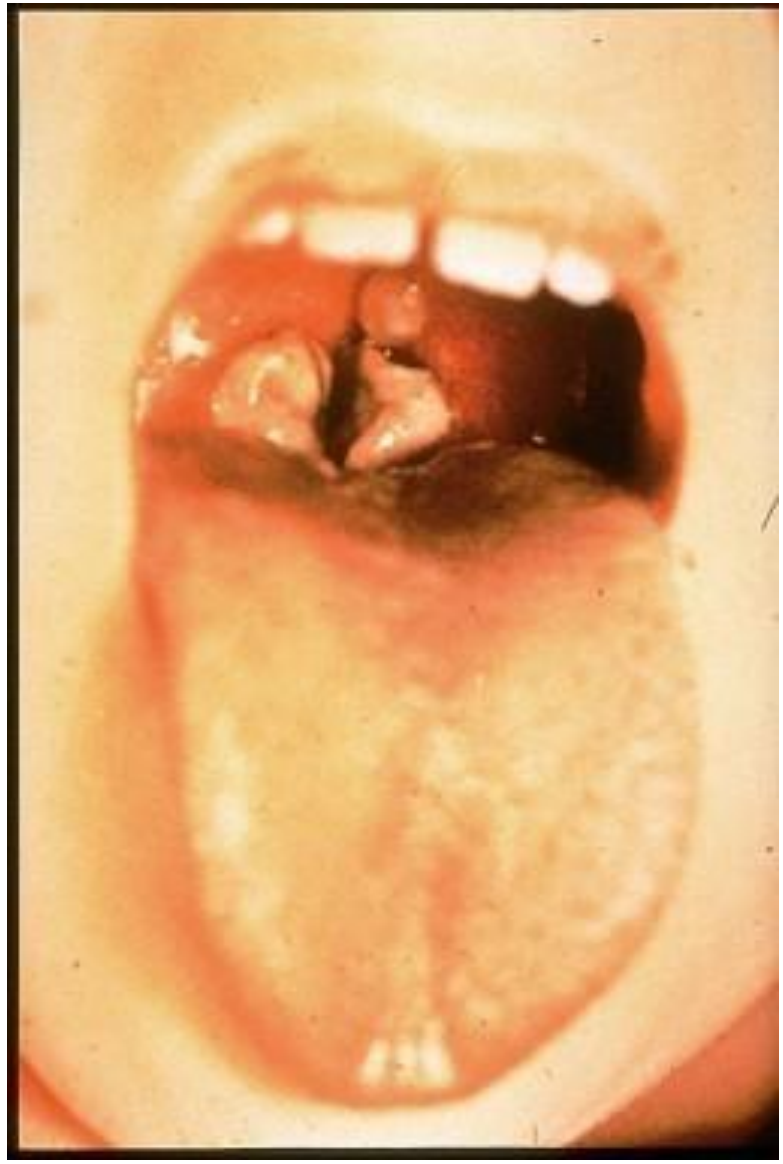
- Prostration and dyspnea soon follow, which may lead to suffocation if not promptly relieved by intubation or tracheotom

- Diphtheria is Lethal disease , solved by:
- 1.putting patient under respirator 2.tracheal intubation

- Damage to the heart causes irregular cardiac rhythm.

- Visual disturbance, difficulty in swallowing and paralysis of the arms and legs also occur but usually resolve spontaneously.

- Death usually may be due to asphyxia or heart failure.

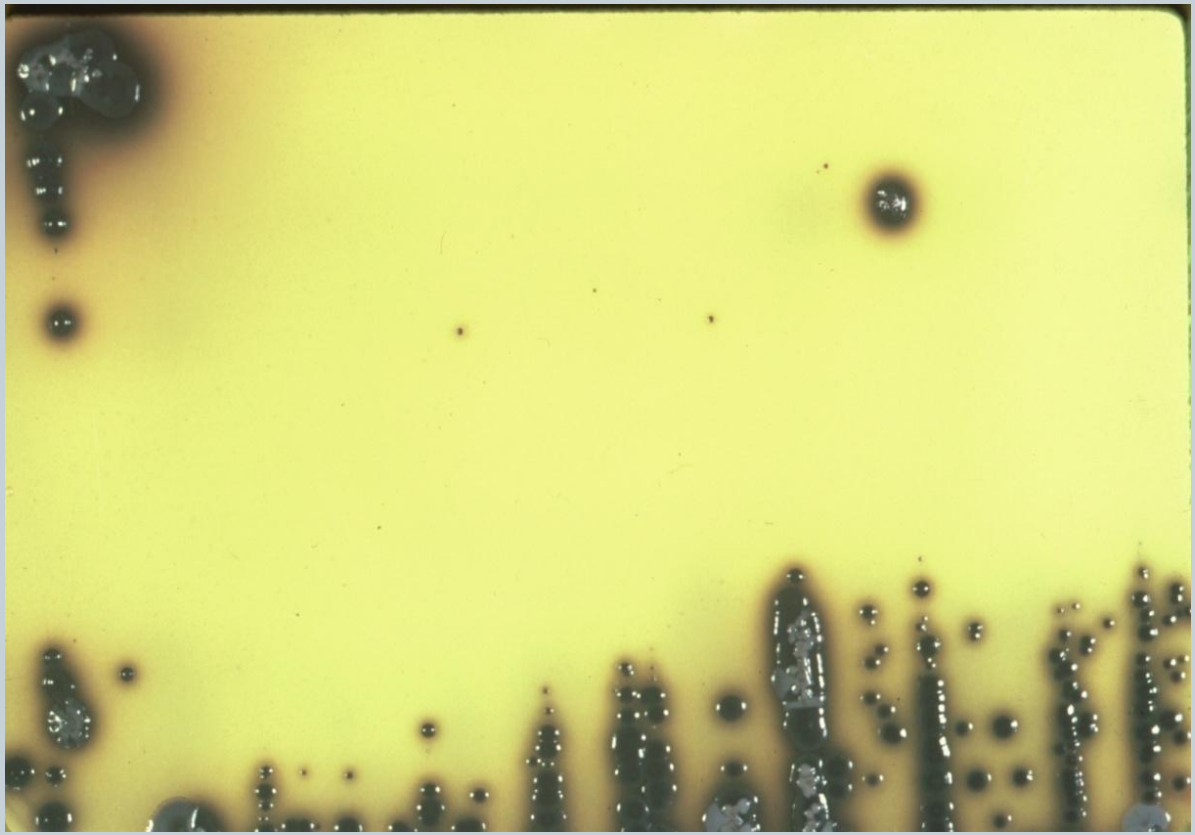


Diphtheritic “Pseudomembrane”

Laboratory Diagnosis

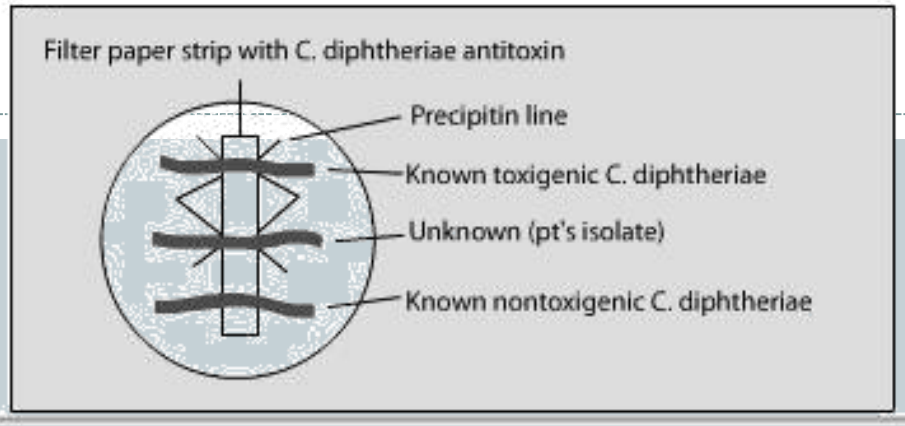
- Diagnosis of This bacteria is easy but treatment is hard
- Specimens: swabs from the nose, throat or suspected lesions.
- Gram's stain: beaded rods in typical arrangement (unreliable).
- Culture: inoculate specimen onto a cysteine-tellurite blood agar plate.
- Identification: biochemical tests (presence of cysteinase).

C. diphtheriae on a cysteine-tellurite plate



Elek Test

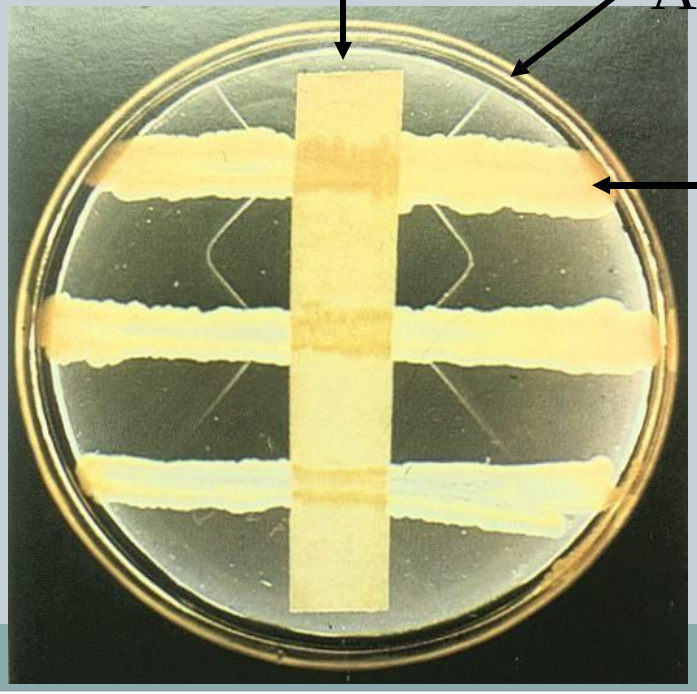
ELEK test:



Elek test is immunological test (Ag-Ab reaction) used to diagnose corynebacterium

sterile filter paper with *C. diphtheriae* antitoxin

Ab-Ag precipitation line



Bacteria

Treatment

- Treatment of diphtheria rests on prompt administration of antibiotics : penicillin, erythromycin (First line) and diphtheria antitoxin neutralization of the toxin (second line)
- Maintenance of an open airway. (by respirator or intubation)
- Treatment of bacteremia or endocarditis must be guided by antibiotic susceptibility tests.

Prevention and Control

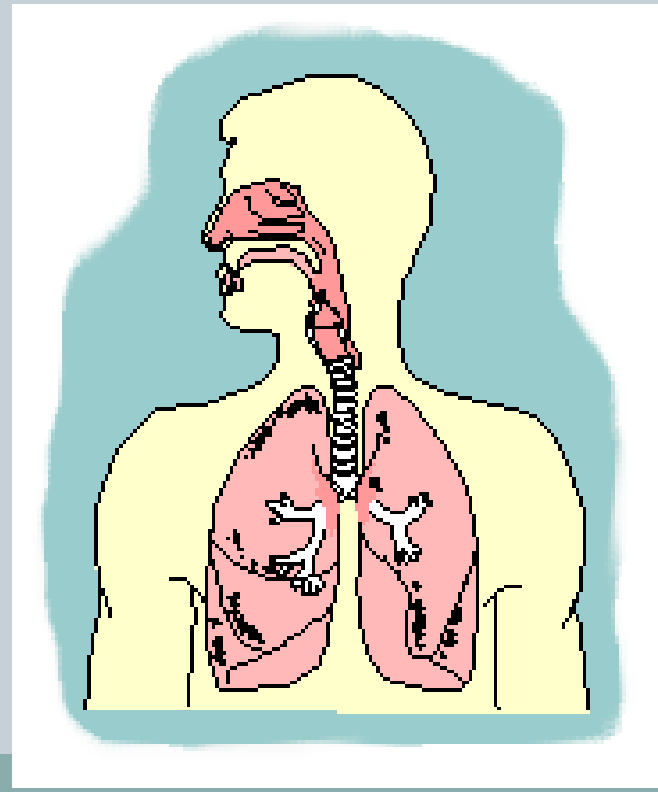
- Humans are the only known reservoir of C. diphtheriae.
- Diphtheria was mainly a disease of small children.
- To limit contact with diphtheria bacilli to a minimum, patients with diphtheria should be isolated.
- Prophylactic antibiotic treatment to unimmunized contacts.

Prevention and Control

- Active immunization in childhood with diphtheria toxoid yields antitoxin levels adequate until adulthood. Usually (DPT vaccine). (Diphtheria,Pertussis,tetanus)
- المطعوم الثلاثي
- All children must receive an initial course of immunizations and boosters (5 injections at 2 months, 4 months, 6 months, 15-18 months, and at 4-6 years).
- Regular booster (every 10 years)

Respiratory viruses

Dr. Waleed Al Momani
Associate professor
Medical microbiology
Faculty of medicine
Yarmouk uninersity



Influenza virus

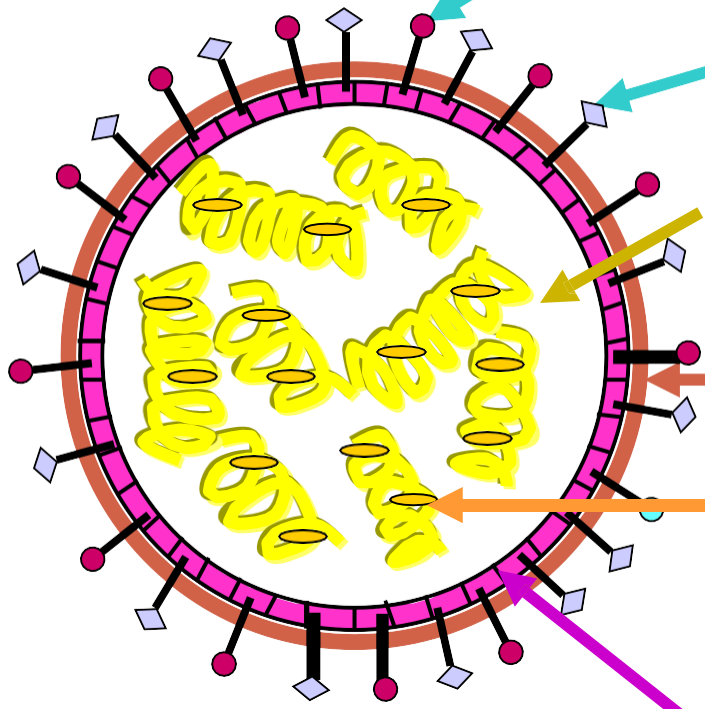
Influenza is a disease caused by influenza virus, member of the orthomyxovirdae

Not to be confused with *Haemophilus Influenzae* which is a type of Bacteria that can't cause Influenza or flu

A common problem in viruses especially influenza virus is the variable antigenicity, every year this virus appears with new modified antigens, so pathogenesis will differ as well as vaccines as older vaccines won't be effective

Stable Antigen : antigen that will remain the same without changing over time

ORTHOMYXOVIRUSES



HA - hemagglutinin

NA - neuraminidase

They are variable proteins (antigen) with new versions every year

lipid bilayer membrane

polymerase complex

M1 protein

type A, B, C : NP, M1 protein

sub-types: HA or NA protein

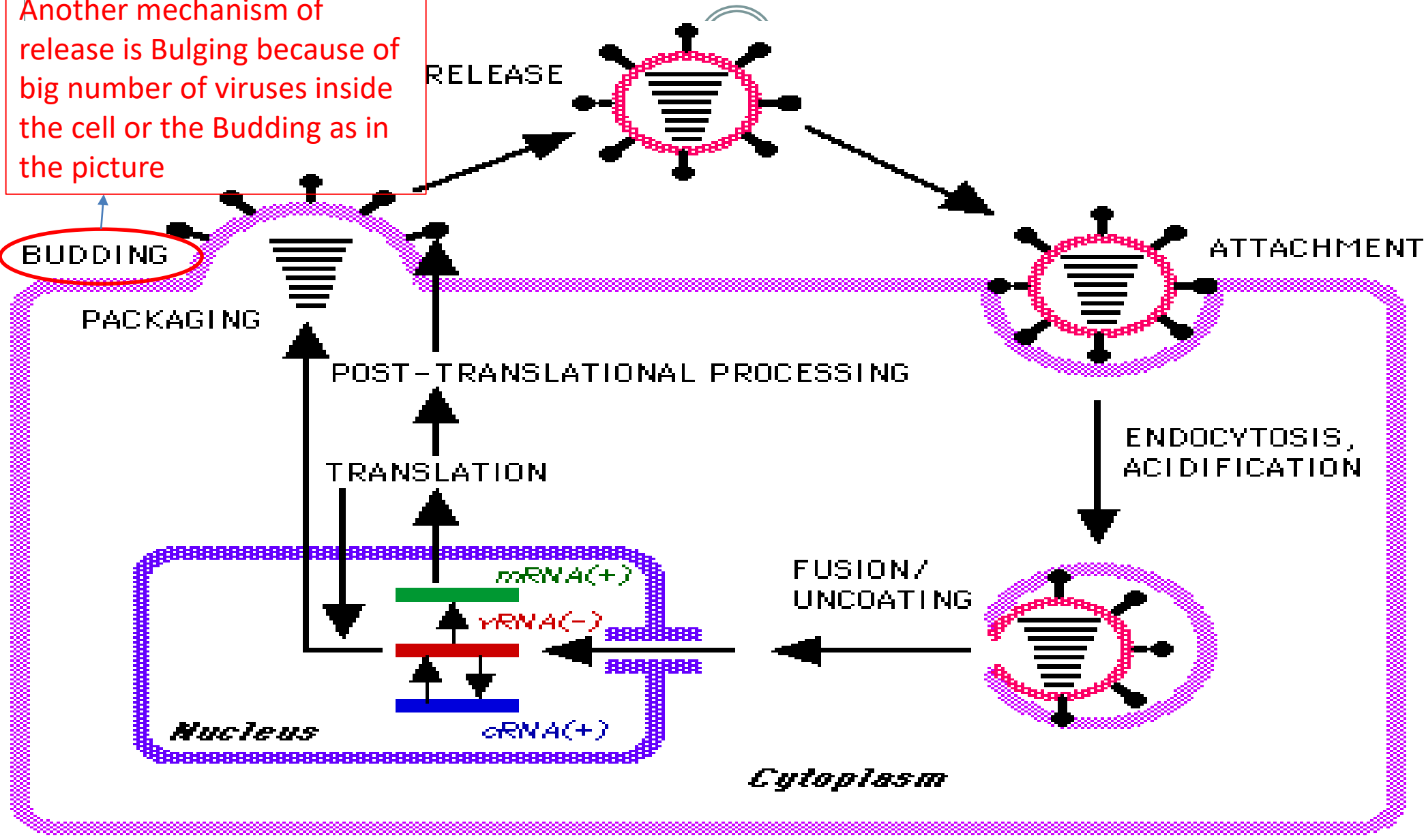
M1 protein (a stable antigen) is the most important part of virus structure

Replication

- Influenza transcribe and replicates its genome in the target cell nucleus
- assemble and buds from the plasma membrane
- Viruses are intracellular particles with no ability to replicate so it uses the genetic machinery of host cells to replicate inside it

Influenza virus

Another mechanism of release is Bulging because of big number of viruses inside the cell or the Budding as in the picture



Antigen

- Soluble antigens: include ribonucleoprotein and M protein which are much stable in antigenicity.

- Surface antigens: include HA (hemagglutinin) and NA (neuraminidase) which are much variable in antigenicity.

Types

- Influenza viruses are divided into 3 groups determined by the ribonucleoprotein (RNP) antigen and M antigen:

the most important type, it has intermediate hosts such as animals, increasing its transmission to Humans

- Group A - This group is the cause of epidemics and pandemics and has an avian intermediate host (IH)

- pandemic: is an epidemic of disease that has spread across a large region; for multiple continents, or even worldwide
- epidemic: is the rapid spread of infectious disease to a large number of people in a given population within a short period of time

- Group B - This group causes epidemics and has no **IH**

intermediate host

- Group C - This group does not cause epidemics and causes mild disease

Most severe and infectious

	TYPE A	TYPE B	TYPE C
severity of illness	++++	++	+
animal reservoir	yes	no	no
human pandemics	yes	no	no
human epidemics	yes	yes	no (sporadic)
antigenic changes	shift, drift	drift	drift
segmented genome	yes	yes	yes
amantadine, rimantidine	sensitive	no effect	no effect
zanamivir	sensitive	sensitive	
surface glycoproteins	2	2	(1)

Subtypes

- According to antigenicity of HA and NA, influenza virus is divided into subtypes such as HnNm(H1N2, et al)

- Three hemagglutinins (H1, H2, and H3) and two neuraminidases (N1 and N2) appear to be of greatest importance in human infections.

H1,H2,H3,N1,N2 are for humans but if the virus came from avian it could differ as H1N5,H2N9…..
And these viruses are problematic, as the vaccines and treatments used for human viruses are not effective

- A/Texas/1/77(H3N2)

How viruses are classified ?

- A/Bangkok/1/79(H3N2).

Group of influenza/city or place/number of isolates/year of isolation/H,N interchange