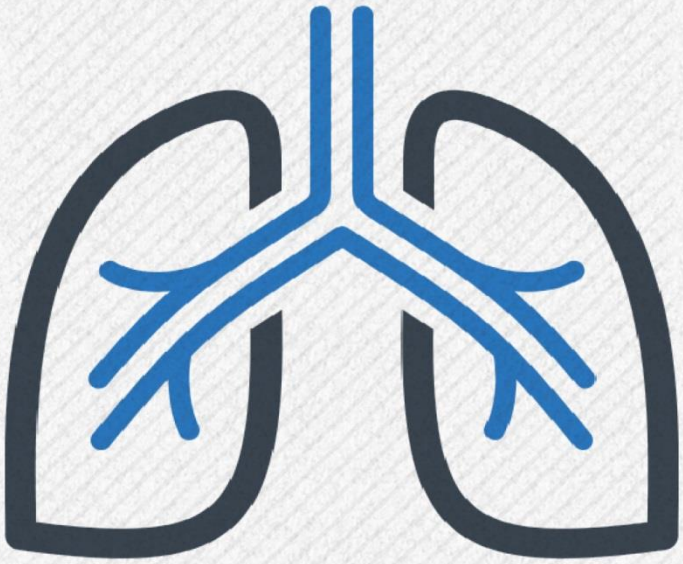

Respiratory system

Microbiology

sheet handout slides

Number	2
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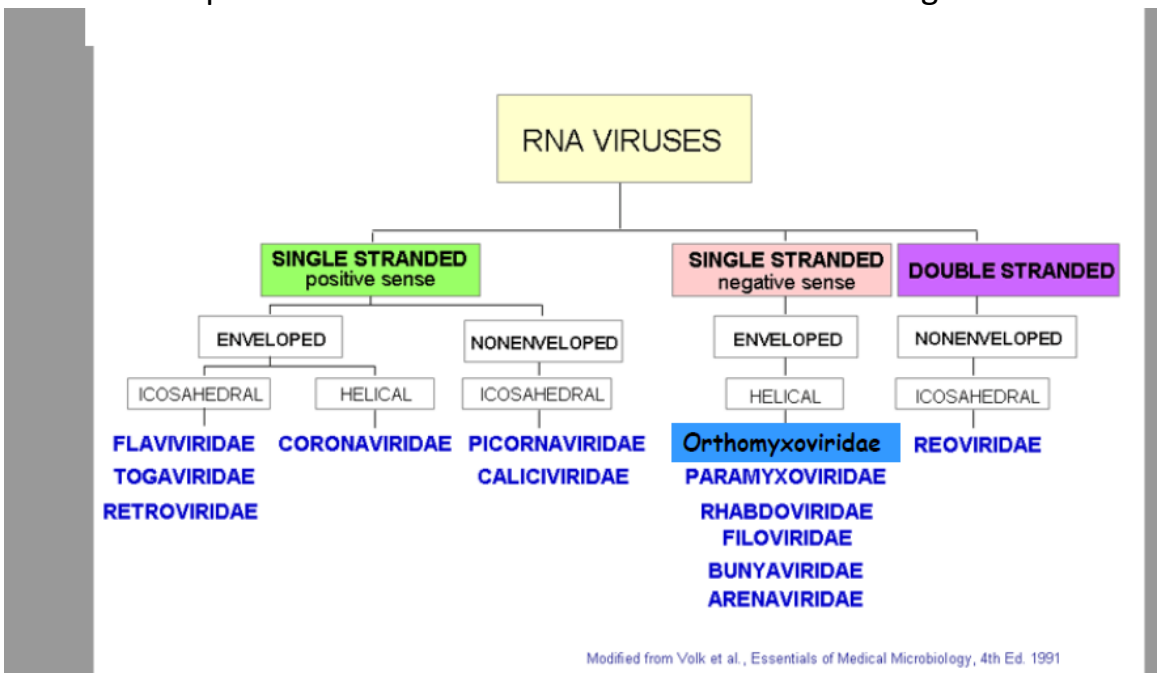
Todays lecture will be about Influenza virus. (this sheet is written with the help of last year sheet)

- Don't look at pages number , it is very easy and comprehensive .

NOTE :- read point number 1 below then come back to the figure .



General information about this virus :-

1- Belongs to Orthomyxoviridae ,the virus is segmented RNA virus , single stranded negative sense , enveloped and helical.

*Remember that segmented viruses means that each segment represnts only one gene which encodes one protein .

*** Segmented viruses are :-**

A-Influenza virus.

B-Rotavirus which is a member of Reoviridaefamily .

2- WE have Three humans influenza viruses **:-**

***A, B and C (more detalis in the upcoming slides).**

***Type A is the most virulent one , B is less virulent than A , while C is very mid and associated with mild even subclinical effect .**

***When we talk about human infleuza illness we are mainly talk about type A and B. Type C as mentiond before causes subclinical illness .**

***Usually infleunza vaccine contains two strains of A and one of B .**

3-Each type has a unique protein
A→M2 PROTEIN.
B→NP PROTEIN.
C→HEF protein.

**SO, what is the significance of these proteins ?
- they are related to antiviral drugs , since antiviral drugs work on these proteins.
-amantidine and remantidine work on M2 protein so inhibiting **type A**.

- Oseltamavir & Zanamavir are Neuraminidase Inhibitors (NAIs); They are used against Influenzas **A & B,** they reduce number of viruses that are released by the infected cell (counteract the action of neuraminidase).

- **Antigenic changes continually occur within Type A ,to a lesser degree in type B.**
-Type C is antigenically stable
-Influenza A strains are also known for ,aquatic birds (e.g.ducks, turkeys, chickens, geese), pigs & horses.

-INFLUENZA TYPES B AND C ARE RESTRICTED TO HUMANS.

Condense view on the virus :-

Comparision :-

NOTE:- TAKE A QUICK LOOK THEN RETURN TO IT AFTER FINISHING THE SHHET .

	Influenza A	Influenza B	Influenza C
Number of segments	8	8	7
Host range (It could infect):	Humans & animals (birds, pigs, cattle, fish,..)	Humans	Humans & pigs
Unique structural protein	M2 protein	NEP PROTEIN	HEF PROTEIN
Severity of symptoms	THE MOST VIRULRNT	INTERMEDIATE	MILD (SUBCLINICAL)
It's susceptibility to antigenic changes	Most susceptible (Unstable). It has both changes shift and drift	Less susceptible (Stable but can change. Associated with antigenic drift only	Least susceptible (Most stable).
Spikes	HA and NA	HA and NA	HA

Influenza "A" virus is so subjected to major antigenic changes that cause occasional world wide pandemics when a new subtype of influenza A appears. Between the pandemics, smaller epidemics are scattered in different locations at intervals of 2-3 years . (don't stuck into this point keep reading and you will find the explanation)

-Epidemic: The increase of number of affected people to more than normal ranges in a specific geographical area at a certain point of time.e.g. Influenza in winter in jordan.

-Pandemic: The increase of number of affected people to more than normal ranges at a certain point of time globally(the graphical region is not confined to a certain area).

Properties of Orthomyxoviruses:

•**Mutability and high frequency of genetic reassortment are characteristics of orthomyxoviruses, two types of genetic mutation are known :-**
–**Antigenic Drift.**
–**Antigenic Shift.**

1- "*Antigenic Drift*" →(associated with epidemics)

•**They result from as little as A SINGLE MUTATION IN THE VIRAL RNA,** due to the presence of an enzyme that lacks proofreading, which is "RNA-dependent RNA polymerase **which leads to gradual changes of antigenic properties of the strain.** (the shape of the glycoprpteins which found on its surface will be changed gradually at each time a mutation occurs).

-NOTE:- gradually means that the new one does not resemble the origin one 100%, it might resemble it with 80-90 % .

- A mutaion will occur every 10,000 bps , and they can happen in any part .
- These mutation of great importance if occur in the glycoproteins of the virus (HA and NA ,espically in HA glycoprotein) because they found in the outermost part of the virus and in direct contact with the immune system .
- Neutrilazing antibodies **are mostly** associated with HA glycoproteins.

- the shape of the glycoprotein change gradually at each mutation so our B memory cells will recognize the new glycoprotein but with <u>less affinity</u> as a result the antibodies secreted will neutralize these glycoproteins with 80-90%. (because the shape of the glycoproteins has changed).

- Once a person transmit the virus to other person , the acceptor person will not have the same form of the virus which was found in the orginal person and thats beacuse of antigenic drift (the shap of HA has changed). (The same idea)

- Every 2-3 years we have epidemic outbreaks.

2-"*Antigenic Shift*". →(associated with pandemics)

- occurs when one cell of human , avian or pig is infected by different species of influenza viruses , so each one of these species will replicate its genome , and once assembly occurs different genomic segments of **different species will assemble in one virus result in a new virus strain .**

***The dangerous risk of this process is that, if we have a new segment of HA glycoprotein introduced to this virus , because it will be a new one and our immune cells do not have a memory cells for it .**

*** So we conclude that antigenic shift is more dangerous than antigenic drift ,and that's , because in antigenic shift a newly virus with new antigenic proteins is produced, so it is hard for our immune cells to fight them , while in antigenic drift although we have a point mutation but the shape of the antigenic proteins do not differ that much from the original one ,so our immune cell are capable to fight them .**

-Influenza "A" virus is associated with antigenic shift and drift , while **type B is associated with antigenic drift only . type C is stable .**

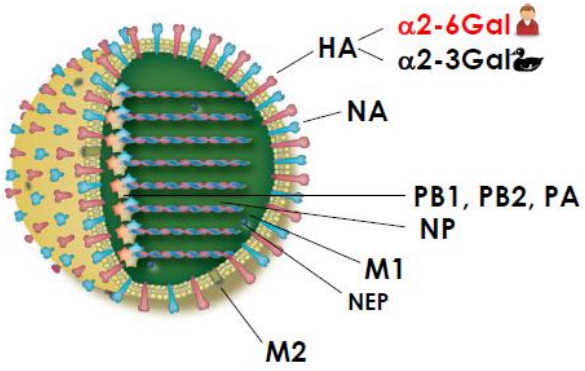
-Every 7-10 years we have outbreak which is more sever and dangerous than those related to outbreaks of antigenic drift .

- **Antigenic shift is more virulent than antigenic drift**

***Influenza is an acute upper respiratory tract infection that usually occurs in epidemics , but also it can cause lower respiratorty tract infection(LRTI) as well.**

-LRTI can be due to primary infection or due to a complication of (URTI) which allows bacteria or other viruses to infect (LRT).

- Spherical virus, (filamentous forms occur).
- Helical nucleocapsid
- Segmented single stranded RNA(eight segments), to which protein capsomeres are attached.
- Enveloped
- Two virus encoded glycoproteins are inserted into the envelope, and are exposed as spikes: HA & NA



-Influenza virus targets **sialic acid recptors** on the host cells .

- Sialic acid receptors which found in our cells are of two types :-

1- alph2-6Gal: is associated with the seasonal infection (mild infection) which infects URT
2- alpha2-3Gal: is associated with "across species infection" which related to lower repiratory tract infection (serous infection). It's more sever with higher mortality.

-Influenza virus replicates in the **nucleus .**

- all RNA viruses replicate in the cytoplasm except influenza and HIV ,

1-Haemagglutinin spikes (H or HA):-
-**So far 15 antigenically different haemagglutinin subtypes exist (H1- H15), and they are strain specific.**

-HA protein binds viral particles to susceptible cells, and is the major antigen against which neutralizing (protecting) antibodies are directed.
-**It derives its name from its ability to agglutinate erythrocytes.**

-Encoded from segment 4.

- H1, H2, H3, H5, H7 are found in humans. The rest are found in different animals.

-H1, H2, H3 and H5 are the most important.

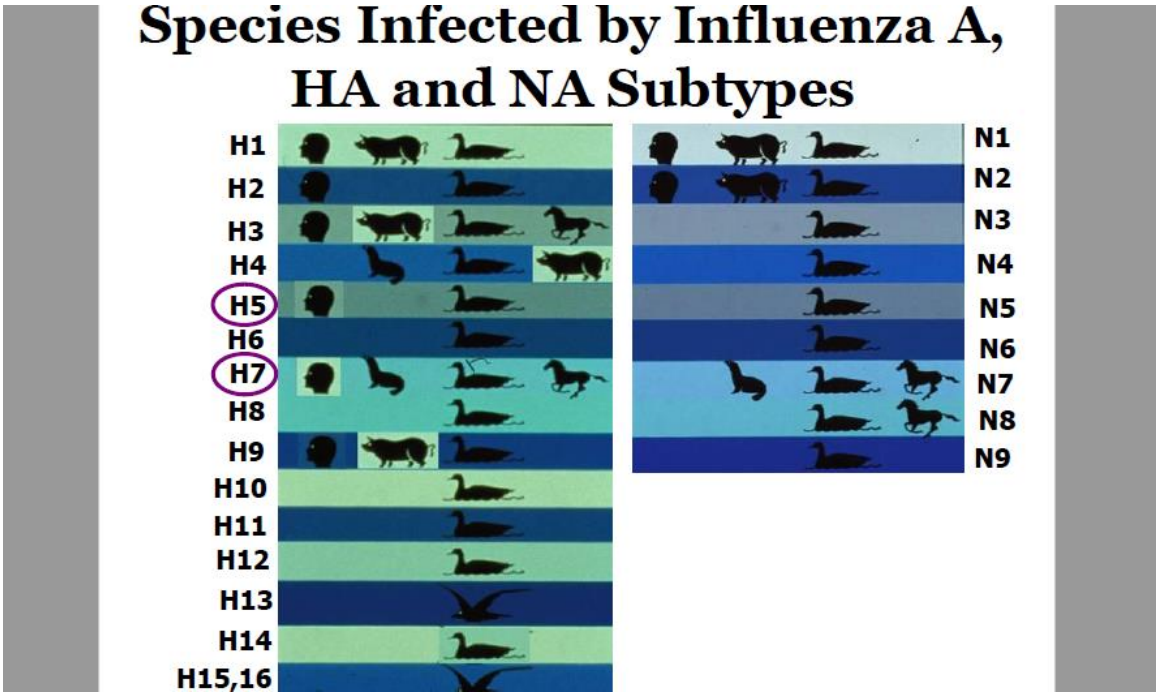
-Amantidine & Rimantidine are used in treating Influenza A,because they work on the uncoating step, specifically at the **M2 protein.**

2- Neuraminidase spikes (N or NA):

-They are mushroom shaped protrusions, antigenically distinct from haemagglutinins.
-At least nine antigenic types exist (N1 –N9).
- They have a role in Inactivation of a free mucoprotein receptor in respiratory secretions .
- plays a role in the Fusion of viral envelope with the host cell membrane (internalization of the the virus into the cells).
-It also facilitate the release of viral particles from infected cell surfaces during budding, and prevent self aggregation of virions.(the clinical importance of this point is in the below point).

-Oseltamavir & Zanamavir are Neuraminidase Inhibitors (NAIs); **They are used against Influenzas A & B, they reduce number of viruses that are released by the infected cell (counteract the action of neuraminidase)**
-Encoded from segment 6.

-N1, N2 are the most important
- another function of neuraminidase is inactivation of premucoprotein receptor on respiratory secretions .



Influenza virus strain designation :- (Naming of the virus)

to name the virus follow the following steps :-
1. **Description of the "S" antigen (A, B or C).**
2. **Host origin:** if isolated from humans we do not mention that is a human type , but it should be mentioned if it isolated from non-human hosts (avian, equine, swine, etc).
3. **Geographical origin.**
4. **Strain number and year of isolation**.
5. **Antigenic designation of the haemagglutinin and neuraminidase i.e subtype.(for type A)**

Examples:
1-A/ Hong Kong/ 1/68 (H3, N2). >>> here it is a human type because we did not mention that it is a human type. 68 refers to the year of isolation (1968) , 1 refers to strain number .
2-A/Swine/New Jersey /8/76 (H1, N1).

3-A/Turkey/Wisconsin/1/66 (H5, N2).
4-A/Poultry/Hong Kong/1/97 (H5,N1)
NOTE:- in examples 2,3,4 we mentioned the host of origin , but if it of human origin we don't mention it .

Influenza virus strain designation

Description of the "S" antigen	Host origin	Geographical origin	Strain number	year of isolation	Antigenic designation of the haemagglutinin and neuraminidase
A	----*	Hong Kong	1	68	H3, N2
A	Swine	New Jersey	8	76	H1, N1
A	Turkey	Wisconsin	1	66	H5N2
A	Poultry	Hong Kong	1	97	H5N1

* Human origin

Avian influenza A virus (H5N1) :-

*The first documented infection of humans by this virus occurred in: 1997 in Hong Kong.
*The source was domestic poultry.
*Back then when the pandemic has appeared, all the poultry were killed in order to eradicate the virus and stop the pandemic.
*All the 8 segments of this virus are from avian origin (no human backbone)
So, it cannot be transmitted from human to human, it jumped across different species

***NOTE:-When a strain of virus jumps across different species, it will cause a pandemic of more pathogenic infection causing a lot of mortalities. Associated with infection of LRT through alpha2-3Gal.**

-With the exception of the Hong Kong outbreak 1968 ,all human pandemic strains have been re-assortants between avian and human influenza viruses.

Pathogenesis of influenza :-

*Transmission: by aerosols/droplets as a result of sneezing or coughing in a confined space
*Replicates in the RS tract, leads to desquamation (shedding) of mucus-secreting and

ciliated cells (so the virus mainly targeting the upper respiratory tract) ,so it's called **" acute lytic infection "**

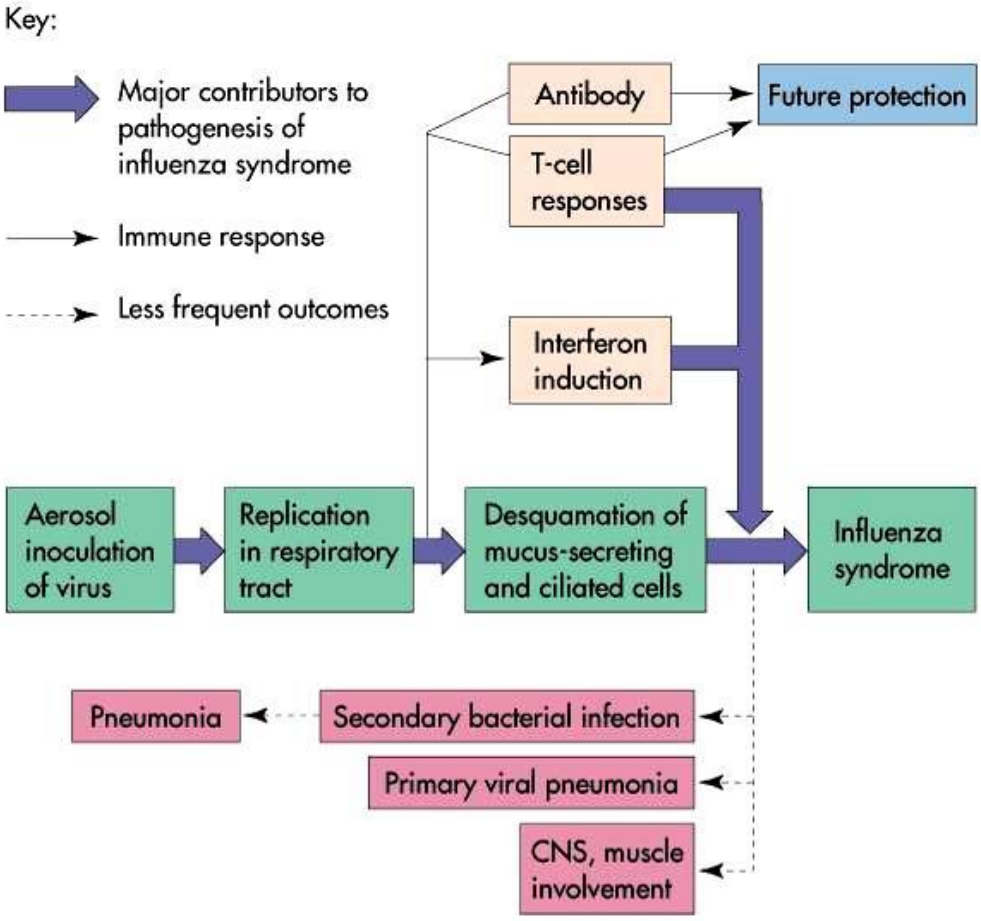
*The symptoms include: high-grad fever ,coughing , sneezing ,runny nose (rhinorrhea & rhinitis), sneezing, general fatigue, arthralgia, myalgia

Note: Conjunctivitis (red eyes) is associated with adenovirus more than influenza virus

- what is the difference between influenza and common cold ?

ANSWER:- influenza is more severe which requires a rest for the patient while common cold is less severe than influenza and does not require a rest .

*VERY IMPORTANT NOTE :- **As a general rule, viral infections can cause a transient drop in immunity; therefore Influenza can lead to superimposed infection with bacteria or infect the lower RS tract (pneumonia**).



Virulence factors

*Ability to infect lower respiratory tract.

*A strong induction of pro-inflammatory cytokines & chemokines (cytokine storm).

*Apoptosis induction.

*Systemic infection.

*Evasion of innate immune response (IFN).

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Figure 1 shows normal respiratory tract with cilia.

Figure 2 shows the respiratory tract after 3 days, acute lytic infection caused the death of cilia.

➔ The cilia require 6-8 months in order to grow back to normal.

** human body will compensate for the loss of cilia by coughing .

Clinical findings

- It's more severe in immunocompromised patients (very young & elderly people) and in people with heart or lung diseases.

A- Pulmonary complications:
1) Croup (Acute laryngotracheobrochitis)
2) Primary pneumonia
3) Secondary bacterial infection (S. pneumonia, Staph. aureus or H. influenza)

B- Non-pulmonary complications:
1) Myositis (rare)
2) Cardiac complications (rare)
3) Encephalopathy (rare)
4) Reye's syndrome (Liver & CNS)
5) Guillian-Barré syndrome

Reye's syndrome
-Hepatoencephaloathy; fatty depositions in the liver & brain.
-Characterized by: brain edema, vomiting, lethargy, coma.
-Seen mostly in children due to treating fever by Aspirin.

It used to be common, but it's rare now due to the presence of other drugs that can treat fever.

-NEVER EVER PRESCRIBE ASPRIN FOR CHILD WITH FEVER .

Diagnosis:-

**It's a clinical diagnosis (based on the clinical picture of the patient);
This has to do with the season (more in winter), symptoms & the signs that the physicians notice.

**Procedures like (Isolation, Serology by ELISA, complement fixation, haemagglutinin are used only in cases of pandemics/outbreaks to identify and sequence the virus .

*Once the virus is identified, these procedures are not needed anymore).

Treatment of Influenza

*Influenza is a self-limiting disease; it can heal alone.
*Symptomatic treatments are routinely made such as: Vitamin C , Chamomile.
*Antipyretics (for fever), Antitussives (for cough), Decongestants (for runny nose), and the most important one is bed rest.

Only in severe cases, we use anti-viral drugs such as :

-Amantidine
- Rimantidine
- Oseltamavir (Tamiflu)
- Zanamavir

Amantidine & Rimantidine are used in treating Influenza A, because they work on the uncoating step, specifically at the M2 protein.

*** Unfortaneutally resistance aganist Amantidine & Rimantidine is about 95%.

Oseltamavir & Zanamavir are Neuraminidase Inhibitors (NAIs); They are used against Influenzas A & B, they reduce number of viruses that are released by the infected cell (counteract the action of neuraminidase).

*Prophylactic treatment: it's given to family members in case one of the family is infected & in case of an outbreak/pandemic

****Prevention**
How can we prevent the influenza virus from spreading?
*Prophylaxis with anti-viral drugs as we mentioned.

*Vaccines (3 types of human vaccine; A, B, C)
- If you take the vaccine, you can still be infected with influenza in the same year. Why is that? It's because of the antigenic drifts & shifts that lead to development of new strains of influenza virus yearly.

- Vaccines are most helpful and recommended for elderly people with lung or heart diseases .

- live attenuated vaccines can initiate an infection in some individuals , while killed vaccines never ever cause an infection .

These vaccines are produced according to our previous knowledge, depending on the geographical region; some countries kept track and studied strains that caused pandemics in previous years.

TYPES OF VACCINES :-

- **Trivalent vaccines (2 A strains, 1 B strains) the most common used .**

- Nowadays, Quadrivalent vaccines (2 A strains, 2 B strains) are being used.

- Today's vaccine has the strains of the pandemics of 2013, 2011, 2009 (H1N1).

*2 forms of vaccine:

1- Intranasal (Live-attenuated) :- the virus will infect the URT cells, and it will mimic the true infection but with less severity .
2- Shot Intramuscular (killed virus) that is given in the deltoid muscle.

How is the live-attenuated (intranasal) vaccine prepared?
1) We bring glycoprotein segments from the 2 A viruses & 1 segment from the B virus.
2) We mix all these 3 in a new virus (once it replicates it will express glycoproteins of those 3 viruses).
3) We allow this virus to replicate in a chicken's egg at a temperature of 34-35 °C incubator.
4) We reduce the temperature into optimal temperature (24°C), this will weaken the virus, although it will stay alive to replicate & infect but NOT cause illness.

NOTE :- (You should ask the patient if he has allergy from eggs before giving him the vaccine because they might get an anaphylactic shock.Some patients have a little allergy that they will only develop hives(جلدي طفح) these are given the vaccine but with caution).

NOTE :- Live-attenuated vaccines are contraindicated in immunocompromised patients.

How is the injectable vaccine prepared?

They might use the same procedure if live-attenuated vaccine or they allow the A viruses alone & the B virus to grow alone, then they combine them together.

- Once the virus is injected, the virus will break and release all its antigens, although our body will mainly produce antibodies against the glycoprotein antigens.

When is the vaccine given?

A new influenza vaccine is developed every year, so once it's released it can be given.

In the USA, they start giving it in July, but the best time to give it to get the maximum benefit is in (August, September, October).

GOOD LUCK