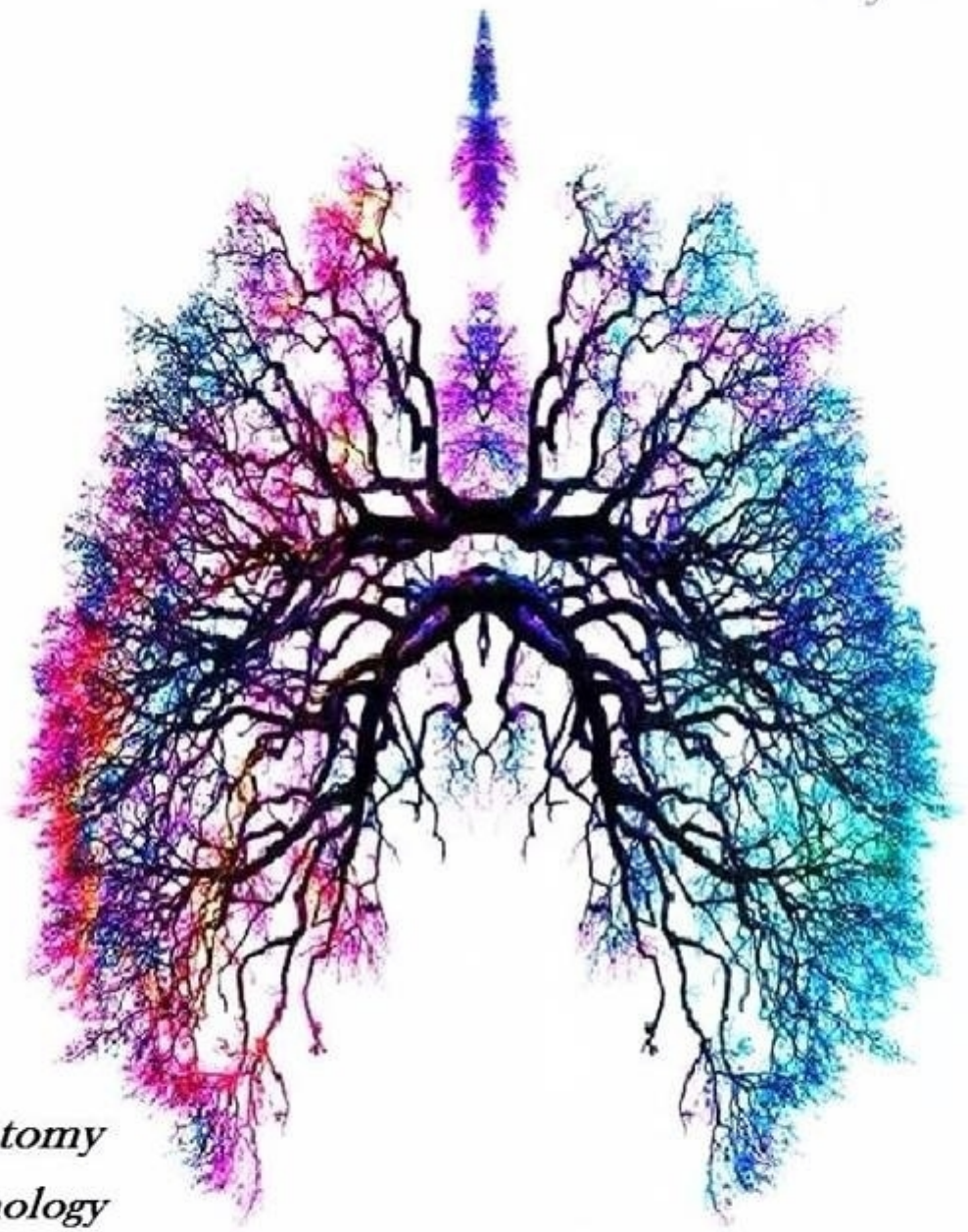
RESPIRATORY SYSTEM

Cover by: Aseil Khatib



- ○ Anatomy
- ○ Pathology
- ○ Physiology
- ○ Pharmacology
- ● Microbiology
- ○ PBL

Dr Name: Dr. Ashraf
Lecture # 3
Done By: Doa'a Dahboor

Sheet ■
Slide □
Other □

Influenza virus

** Sheet references: Lecture's recording and Medical Microbiology by Greenwood – 18th edition – The sheet was written without the slides being provided. At some points, the doctor was reading QUICKLY from the slides. I couldn't write every single word he read, so please refer to them once they're available. I focused on clarifying concepts.**

Influenza virus belongs to the **Orthomyxoviridae** family which has an **RNA** genome. It's an enveloped virus with a **helical capsid**. There are three types of the virus: influenza A, B and C.

Influenza A virus is subjected to major antigenic changes that cause occasional worldwide pandemics and new subtypes of influenza A appear. Between the pandemics, (what is this 7:28) epidemics are scattered.

As mentioned, influenza virus is characterized by both pandemics and epidemics. Let us define these two:

Epidemic→increase in the number of cases of a certain infection in a certain location at a certain point of time.

Pandemic→ increase in the number of cases of a certain infection globally (worldwide) i.e. global spread of a certain infection.

Also, influenza virus is characterized by continuous variation in its genome. This is related to two distinctive features:

1. RNA dependent RNA polymerase lacks proof reading → introduction of point mutations every 2500-10000 bases as RNA polymerase is doing its job which is replicating the genome of the virus. This is labeled as **antigenic drift** which is associated with influenza epidemics. This is what we see every winter where there's a seasonal increase in influenza cases (annual increase) – Further clarification for this in page 3.

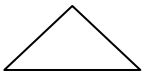
2. Antigenic shift (aka **re-assortment**) → Occurs when a certain cell becomes infected with more than one species of the virus creating new combinations of viral gene segments. By different "species" we don't mean a combination of influenza A, B and C , "species" here refers to different influenza types that affect different hosts. Examples include: human influenza virus, avian influenza virus, swine influenza virus, horse influenza virus, etc.

When a cell becomes infected with more than one of these influenza virus species, this results in new combinations between the genomes of these different species and this is the concept of "re-assortment or antigenic shift". Example: by human and avian influenza virus, by human and swine influenza virus, by human, swine and avian influenza virus, etc.
Antigenic shifts are associated with influenza pandemics with more severe symptoms than those accompanying influenza epidemics .The last reported influenza pandemic was in 2009, it was caused by H1N1 re-assortment virus.
Let me summarize it for you again by this table ☺

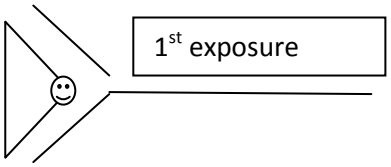
	Antigenic drift	Antigenic shift (Re-assortment)
Entity / definition	Point mutations due to lack of proof reading.	Genetic combinations of different species (human, avian, swine) Infecting the same host.
Epidemiology	Epidemics	Pandemics (global)
Degree of genetic variation	Less	More
Symptoms	Less severe	More severe

- ❖ Why do antigenic shifts cause pandemics with severe symptoms, whereas antigenic drifts cause epidemics with less severe symptoms?

As you know, the glycoproteins of the spikes are the most antigenic part of the virus. These glycoproteins differ in different species i.e. glycoproteins of avian influenza virus differ from those of human or swine influenza virus .Let's compare the two scenarios:

First case: *Antigenic drift:*
Suppose that this is the antigen (glycoprotein) → 
A human that has been infected by a virus having this glycoprotein will develop antibodies against the glycoprotein to neutralize the virus and prevent it from attaching to its receptor on target cells.

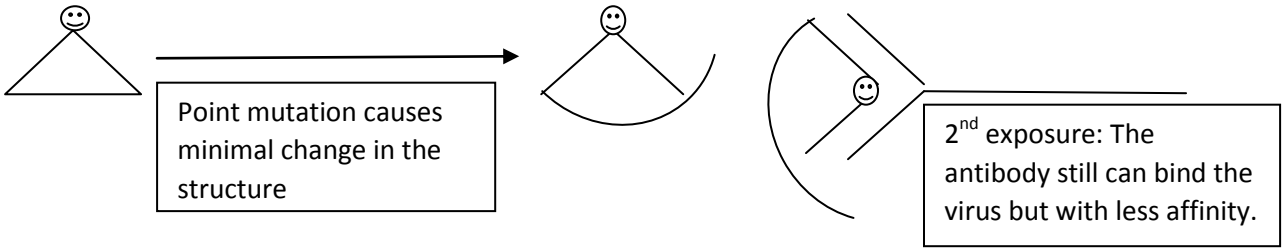
This is the antibody- antigen complex:



The point marked by the smiley face ☺ is where binding mostly occurs; we are concerned with this part of the antigen more than any other part.

Point mutations can occur anywhere in the genome of the virus i.e. can end up affecting structural and non-structural proteins. However, affecting structural proteins is more significant especially those mutations affecting glycoproteins.

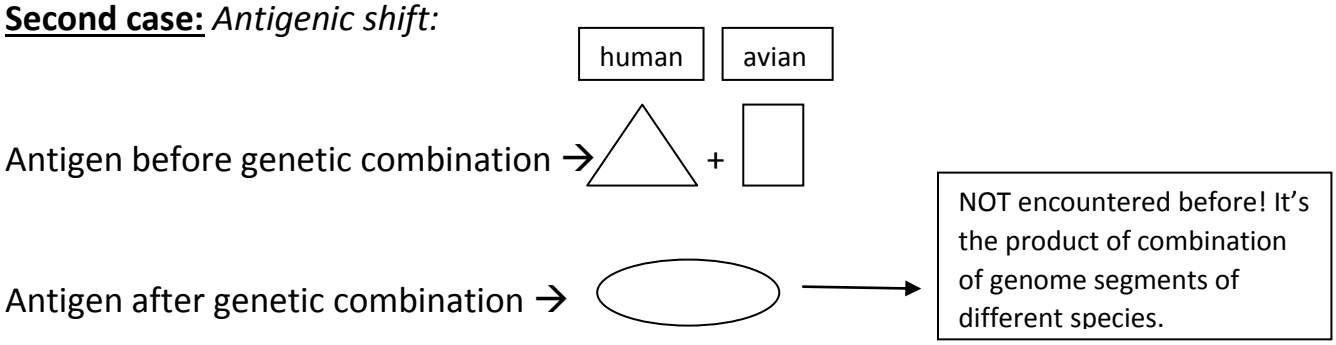
Suppose that a point mutation affected the glycoprotein as follows:



The part that concerns us (marked by the smiley face in the previous drawing) is not affected i.e. the antibody can still bind the antigen and neutralize it BUT with less affinity. Let's say the affinity reduced from 95-99% before the mutation occurred to 80-90% after the mutation. Only 10-20% of the virus will escape neutralization and cause symptoms that accordingly will not be that severe. So in antigenic drifts, the patient has some kind of immunity against the virus since he has been exposed to it previously. Also, the genetic variation is represented by point mutations that do not alter the structure massively thus it can still be recognized by the antibodies formed due to a previous exposure to the virus.

Note :The affinity may reduce from 95-99% to 20-30% if the ☺ was involved and changed by the point mutation, symptoms will be more severe since more virus particles will escape immunity .

Second case: *Antigenic shift:*



Notice the massive change in the structure of the antigen after the recombination. Here we have a JUMP from animal to human due to genetic combination of different species of influenza. The human has never been exposed to such an antigen and there is no previous immunity! Most of the virus particles will escape neutralization and enter the target cells to replicate there . Accordingly symptoms will be so severe ☹

Antigenic drifts → Production of a more virulent virus, affinity for the pre-formed antibodies drops from 95% to 20-30% approximately, occurs in epidemics every 3-5 years.

The doctor here clarified something: There's increase in the number of influenza cases annually (annual epidemics in winter months), this doesn't necessarily mean there's a production of a more virulent strain by an antigenic drift (changing genome by point mutations) every year. Indeed, a new more virulent strain develops by antigen drifts every 3-5 years.

Antigenic shifts → Production of a more virulent virus strain, occurs every 7-10 years as pandemics, the last one was in 2009.

❖ Where did the name "influenza" come from?

The name came from the influence of bad air. Previously, they didn't know about the existence of viruses at all! They used to think that bad air comes to certain areas and threatens people's lives there. An example is the 1918 pandemic of Spanish flu that killed more than 20 million people.

Influenza is an acute respiratory tract infection that occurs in epidemics and pandemics due to the previously explained genetic alterations that affect affinities toward antibodies i.e. affect affinities toward mucus.

There are three types of influenza virus: A, B and C. Type C causes very mild illness (considered an asymptomatic infection), so most of the time when talking about influenza virus we mean either types A or B. A unique feature for influenza virus is its segmented genome (in circular conformation) . Each segment encodes one protein only.

Note: Other virus that has segmented genome is rotavirus.

Influenza A and B → 8 segments.

Influenza C → 7 segments.

Type A → Human, avian, swine and horse.

Type B and C →Restricted to humans - at first the dr. said that influenza B may affect swine, I think this is not right : I referred to wiki- : Wiki- says that influenza B affects humans and seals – إنفلونزا ب يصيب الإنسان و الفقمة

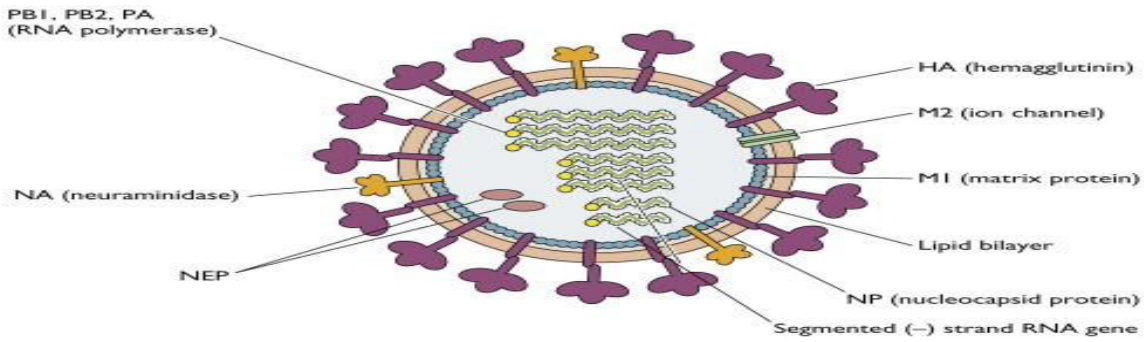
Type A is associated with BOTH antigenic shifts and drifts more than type B.

Type B is associated with antigenic drifts ONLY.

❖ Structure of the influenza virus :

- Spherical virus.
- Helical capsid.
- Segmented ssRNA (7-8 segments that are attached to the proteins of capsomeres).
- Enveloped virus.
- There are two viral encoded glycoproteins that form spikes: The hemagglutinin (HA or H) and the neuraminidase (NA or N).
- Other structural proteins include: M1, M2 and NEP proteins – see the figure below. These proteins are present in certain types of the virus, for example: M2 protein is present in influenza A ONLY.

✓ This is important since it's highly linked to therapy. We have 2 classes of drugs: The first class is neuraminidase inhibitors. These drugs can be used to treat influenza A and B since it depends on inhibiting NA protein which is present in both of them.
The second class of drugs is amantadine and rimantadine. These drugs affect the un-coating step by binding to the M2 protein which is present in type A influenza only, thus these drugs cannot be used to treat influenza B.



H protein: there are 15 H proteins (H1-H15), they're strain-specific. The most important in humans are H1, H2 and H3 .The name hemagglutinin is derived from the ability of this protein (spike) to agglutinate erythrocytes. The primary role of H protein is the attachment to receptors on target cells.

(The dr. then said that at least 9 antigenic types exist. The number differs according to the reference and it's increasing with every research .I referred to wiki- : Wiki- says that H18 was discovered in 2013! I want you to know what is right☺ It's not only about exams.)

The most antigenic glycoprotein is the H protein and not the N. The immune system recognizes and produces antibodies against the H protein to neutralize it and not against the N protein. Why is that? Remember that the primary function of the H protein is attachment to receptors on target cells. If you inhibit this protein, then there will be no attachment to target cells, thus the virus will not enter them and will not replicate.

N protein: has 12 or 13 types. The most important are 1, 2 and to a lesser degree 3. What about its function? The newly-produced influenza virus remains attached to the host cell via the N-acetylneuraminic acid (sialic acid) which is a part of the host cell's recepto. This binding is cleaved by the neuraminidase in order to "free" the virus to infect nearby cells. (Greenwood: This action allows the virus to permeate through the mucin overlying host epithelial surfaces).

Neuraminidase inhibitors block this step i.e. block the release of the virus from the cell.

These proteins have also a role in (functions):

- The fusion of the viral envelope to the membrane of the host cells.
- Inactivation of mucoprotein receptor in respiratory secretions.
- Facilitating the release of viral particles from infected cells during budding and thus prevention of virions aggregation.

❖ **Strain designation (nomenclature) :**

Many things are included in nomenclature:

- the type (A , B , C)
- the host origin (human , avian , swine , horse – when the host is human usually it's not mentioned in the nomenclature , other hosts must be mentioned)
- geographical origin (the first place where the virus was identified/isolated)
- description of the S-antigen
- strain number
- year of isolation
- antigenic designation of H and N (more important to be mentioned for type A influenza , an example is H1N1)

The full name of the virus is usually written on vaccine shots against these viruses. The doctor mentioned an example of nomenclature from the slides , please refer to them once available.

❖ Each RNA segment encodes one specific viral protein thus antigens undergo genetic variation independent of each other.

Segment 4 encodes → hemagglutinin

Segment 6 encodes → neuraminidase

These 2 proteins represent the envelop spikes.

The doctor said again that high frequency of antigenic drifts and shifts (re-assortment) is a characteristic of Orthomyxo-viruses. Re-assortment results when the genomes of more than one species of influenza (human, avian, swine) contribute by different segments in the same host cell. When assembly of the virus occurs, a mixture of these segments from the different species will be incorporated in one new virus ! By this mechanism, human can be infected by avian or swine influenza. Antigenic drifts occur due to lack of proof reading that results in point mutations as explained previously.

The avian influenza A subtype **H5N1**: The first documented infection of human by avian influenza was in 1997 in Hong Kong. In that particular case, there was no mixture of human and avian genome segments, instead, all the avian type segments were involved in virus particles that affected humans, so there was a JUMP from animal to human. Because of this, there was a global pandemic! They found that the infection was acquired through birds only. An infected human is not contagious to the others i.e. transmission between humans was unlikely. At that time, they used to burn birds since they were the source of infection.

Note: All other pandemics included mixture of human and avian genome segments; the 1997 pandemic was an exception with very severe symptoms.

❖ Influenza pathogenesis:

How is influenza transmitted? The primary source of infection is droplets or aerosols (As a result of sneezing or coughing in closed places). Another source of transmission: fomites (things that belong to the patient which are contaminated with his secretions).
Influenza can cause both upper and lower RTIs, but most commonly it causes upper RTIs. In complicated cases, the infection spreads to reach the lower respiratory tract, bacterial super-infection might also occur in this case.

The virus causes desquamation of ciliated cells and mucus secreting cells i.e. influenza is an acute lytic virus. It causes death of upper respiratory tract cells, more specifically, their cilia. As you know, the cilia are responsible for clearing the airways. If the cilia are paralyzed, there'll be no clearance of the airways. The body tries to compensate for this by continuous coughing that persists for several weeks! You might recover from influenza after a few days of infection, however; the cough may persist for weeks since the cilia need 8-12 weeks to return to their original shape. (Cilia are dead by the third day of infection, cilia start to grow once again 7 days after infection, complete growth is achieved few weeks later)

Some consequences of influenza :(Doctor will be discussing them further in a bit)

- 1ry viral pneumonia by the influenza virus (in lower respiratory tract).
- 2ry bacterial super-infection.
- Muscles and CNS might be involved (very rare).

❖ **Immunity :**

The two arms of the immune system are the adaptive and the innate immunity. Both arms play a role in the defense against influenza virus. The adaptive immunity is represented by humoral and cellular immunity.

- The humoral immunity is represented by antibodies secreted from B cells that originate from plasma cells. Accordingly, the humoral immunity is involved when the virus is extracellular; virus particles will be neutralized by antibodies.
- The cellular immunity is represented by T helper cells (CD4 +ve) and cytotoxic T cells (CD8 +ve). The virus will be detected by these cells by the aid of APCs via the major histocompatibility complexes (MHCs). Intracellular viruses will be presented by MHC class 1 molecules to be detected by cytotoxic T cells (CD8 +) that release perforins and granzymes to kill the pathogen. Extracellular antigens will be engulfed by macrophages, represented by MHC class 2 molecules and detected by T helper (CD4 +) cells. Both TH1 and TH2 will be activated. TH1 cells (cellular immunity) mediate their action by releasing cytokines that have systemic effects (these systemic effects may involve mediating innate immunity). TH2 cells activate B cells to release antibodies.

❖ **Symptoms** :

They include Fever, headache, myalgia, cough, rhinitis, ocular symptoms such as conjunctivitis (Remember: Conjunctivitis is highly linked to adenovirus).

Clinical findings (symptoms) are more severe in: immunocompromised patients, the young, the elderly and those with per-existing conditions of the heart and lungs.

❖ **Complications of influenza virus** :

- Croup (laryngotracheobronchitis) – Remember : Para-influenza virus is a primary cause of croup .
- 1ry viral pneumonia by the influenza virus (Lower RTI).
- 2ry bacterial infection (by streptococcus pneumoniae , Staphylococcus aureus and Haemophilus) . Complications of these bacterial infections include otitis media and sinusitis.
- Non-pulmonary complications including myositis , cardiac problems , liver problems , CNS and PNS problems (rarely) .
- Reye's syndrome: In children after treatment with aspirin (rare nowadays due to awareness – aspirin isn't used anymore for viral illnesses in children). This syndrome is associated with: fat deposition in liver, edema in brain, vomiting, lethargy and coma.

❖ **Diagnosis** :

Mainly based on clinical presentation (we care about signs and symptoms, patient's age, time of the year (epidemics in winter)). The investigation this way is not done routinely for clinical diagnosis, it's done very rarely (ex.: in cases of researches about new re-assortant strains). We don't have specialized labs for diagnostic purposes in hospitals and primary care centers.

❖ **Prevention and treatment :**

Vaccines and antiviral drugs are available.

Remember the two classes of antiviral drugs we talked about:

- Amantadine and Rimantadine: Block the un-coating step by inhibiting the M2 protein which is present in influenza A thus these drugs are used to treat influenza A only (Provide 70% protection against influenza A, considered as high-risk, nowadays there's great resistance against them ☹ resistance might reach 90% in certain populations! thus they're not effective anymore, NA inhibitors are more effective than them).
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- Neuraminidase inhibitors (Oseltamivir (Tamiflu) and Zanamivir): Large amounts of Oseltamivir (Tamiflu) were produced and sold during the 2009 pandemic in order to be used as a prophylactic drug against H1N1.

The drugs must be administrated as early as possible (most effective in reducing virulence /severity in the first 48 hours after infection).

There are two forms of influenza vaccine:

- An injected form (shots): Widely used globally, given IM in the shoulder.
- An intranasal form.

An influenza vaccine consists of 3 types of antigens (2 from influenza A and one from influenza B). We design a vaccine depending on our knowledge of the most virulent strains in the previous year(s). For example, an antigen from the H1N1 (2009 outbreak) was incorporated in influenza vaccines till the year 2014.

Let us discuss the difference between the previously mentioned forms:

First: The injected form: Inactivated (killed) virus vaccine. It's made by growing the most three virulent strains , then addition of formalin to kill them thus these virus particles are not able to replicate in the recipient body . After injection of the virus, the person will benefit from viral glycoproteins by adapting immunity against them thus the person is protected against the 3 strains we used in making the vaccine only. The person might get infected by other strains of influenza that are not incorporated in the killed vaccine. Also keep in mind that influenza viruses are continuously subjected to antigenic drifts that alter their structures. You might get infected by one of the viruses that are incorporated in the vaccine after being altered by drifts, in such a case, the altered virus slightly differs from the one you are immunized against and due to this structural difference, the altered virus escapes immunity and symptoms might appear. The doctor says that drifts may occur in the virus while it's replicating in the patient's body i.e. you might be infected by a strain and release an altered one that differs from the one that originally infected you!

Second: The intranasal form: Live attenuated vaccine. Generally, live attenuated vaccines are contraindicated in immunocompromised patients. Previously, they used to grow the attenuated vaccines in eggs. Nowadays, eggs are still be used but cell lines are also used as incubators for the attenuated vaccines. In developed countries, you fill a form before taking the vaccine, one of the form's questions is if you have allergy to eggs since the vaccines are grown in eggs. They take the genome

segments of the most three virulent virus strains , re-assortment of the genome segments is done in order to incorporate them together i.e. to form a “whole virus vaccine” from the genomes of the most virulent strains . This vaccine (the combined virus) that’s composed of genome segments of the three strains will be grown in incubators (the eggs or cells we talked about). In the incubators, the temperature is lowered to a sub-optimal degree (at first they put the vaccine seed strains at 33-34 Celsius then they decrease the temperature till reaching 23-24). Due to these sub-optimal conditions, the virus particles are attenuated (weakened) but still alive i.e. they still can replicate but without causing illness. The vaccine is given to the patient by the intranasal route, the virus “the whole virus vaccine” replicates in the nasal cavity or in the respiratory tract without causing illness, however; the immune system is sensitized to the antigens in the vaccine. It’ll “fight” them and accordingly immunity against the three involved strains is developed.

Vaccines are given yearly starting from July, August and September – these are the best times to take the vaccine (once the new vaccine is produced, they start giving it).

End of the sheet. Feel free to give me your feedback. Good luck.

" إذا سمعتَ صوتًا بداخلك يقول : (إنك لا تستطيع الرسم) ،

فهذا يعني : (ارسُمْ) ،

سيَصمتُ ذلك الصوتُ بداخلكَ للأبد ! "