7

Microbiology

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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Topics of the this lecture :

1) Particles to PFU ratio
2) Single step and multi step growth cycle
3) Multiplicity of infection (MOI)
4) Physical measurement of virus particle

Particle to PFU ratio

-Last time, we said that not all viral particles are infectious. Many of them are not infectious even though they look like normal virus particles.

- So, it makes sense to create a ratio between total number of viral particles and total number of infectious particles (virions).

- Particle to PFU ratio = $\frac{Total\ no.\ of\ viral\ particles}{Total\ number\ of\ infectious\ particles}$

- Some viruses don't follow one hit kinetics (a single particle can initiate infection), and the ratio is not 1:1 for most viruses due to many reasons: some viruses are damaged, mutations take place in them decreasing their infectivity, and their infectious cycle is complicated.
- So you can never know (depending on the virus), if the physical particles are actually having the effect that you are looking at.
- It doesn't really matter if non-infectious viruses exist or not because they don't have any effect ☺
- To count the number of physical particles (infectious + non-infectious), there are two ways:

1) Looking at them by electron microscope and counting them.
2) Using some other assays

-In order to quantify infectious viruses, there are some methods:

1) Plaque assay forming unit (PFU) ⟶ 1 plaque origins from one virion.
2) End point dilution assay.

- The following table shows particles to PFU ratio in some animal viruses:

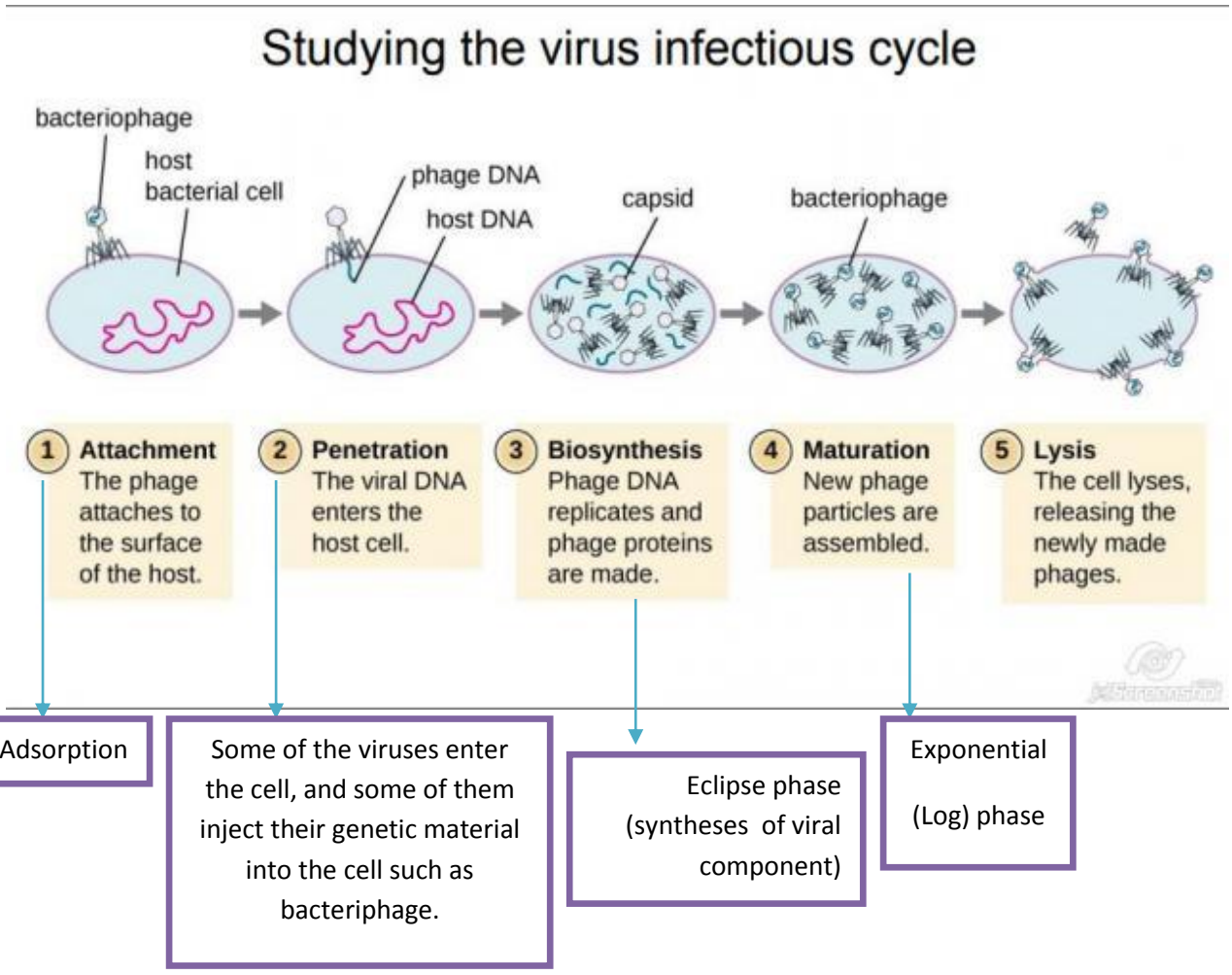
Virus	Particle/PFU ratio
Papillomaviridae	
Papillomavirus	10,000
Picornaviridae	
Poliovirus	30–1,000
Herpesviridae	
Herpes simplex virus	50–200
Polyomaviridae	
Polyomavirus	38–50
Simian virus 40	100–200
Adenoviridae	20–100
Poxviridae	1–100
Orthomyxoviridae	
Influenza virus	20–50
Reoviridae	
Reovirus	10
Alphaviridae	
Semliki Forest virus	1–2

In Papillomavirus, the particle to PFU ratio is 10,000 which means that there is only one infectious virus for every 10,000 particles.

There is one infectious virus for every 1-2 particles.

- The ratio is variable between different viruses.

Particles → (infectious+non-infectious) - PFU → (infectious)



Adsorption

Some of the viruses enter the cell, and some of them inject their genetic material into the cell such as bacteriphage.

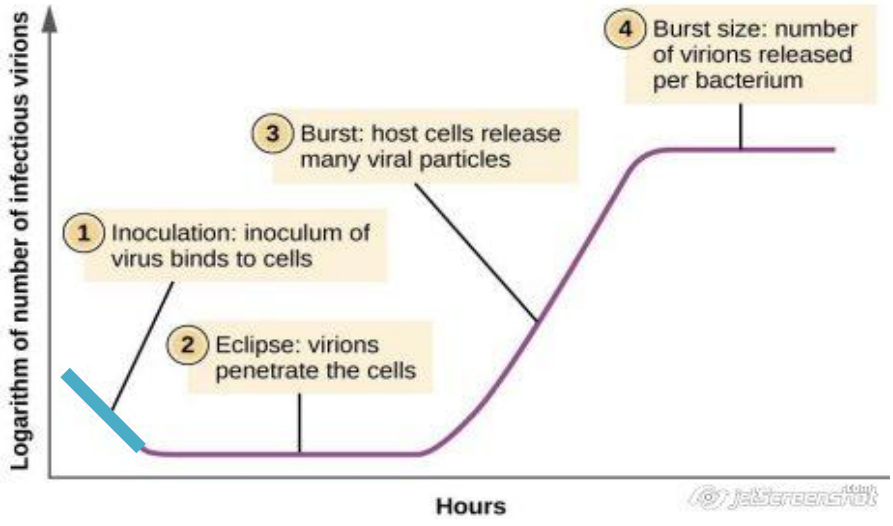
Eclipse phase (syntheses of viral component)

Exponential (Log) phase

-There are two types of the viral growth cycles and each type follows a special curve depending on how much virus initially added into the culture:

1) One step growth cycle
2) Multi-step growth cycle

One step growth cycle



- It was first developed at 1939 by Emory Ellis and Delbruck while working on bacteriophages.

- Take the virus preparation and adsorb it.

-Then the culture is diluted, so no more infection occurs.

-Then the culture is synchronized (all the cells in the culture are in the same growth stage).

-Samples are taken at different times after infection (infectious viruses produced over time).

- Measure virus by plaque assay.

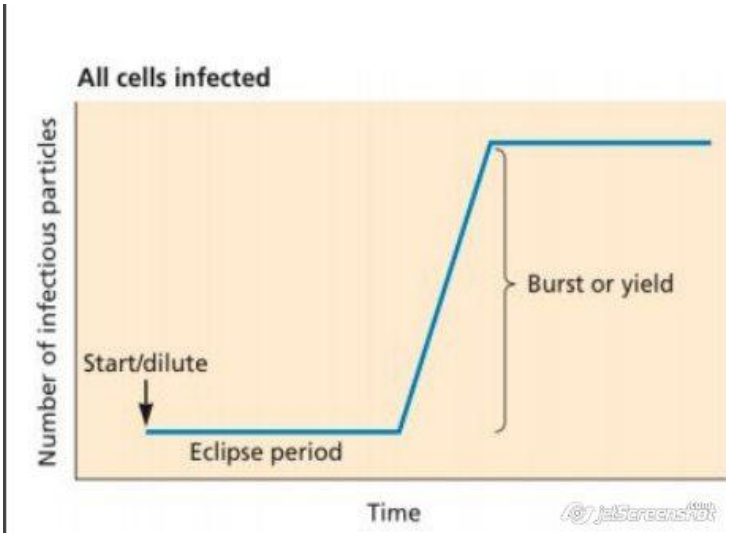
This curve follows phases:

1) **Inoculation phase**: addition of the viruses to the cells.
As you see (in blue), the number of viruses is decreased after inoculation because after infection, the inoculated viruses (viruses bound to the cells or viruses penetrated into the cells) disappear, so the infectious particles can't be detected (not visible when counting), even if the cells are disrupted.

2) **Eclipse phase**: the number of infectious viruses is not changing and remains constant over a period of time because virions have penetrated into the cell. Inside the infected cells, the viral DNA is being replicated, the mRNAs are being produced, the viral proteins are being synthesized and those different components are ready to be assembled together inside the cells exponentially. (**Apparently no infectivity is being generated, but also lot of synthesis in the cell is going on**).

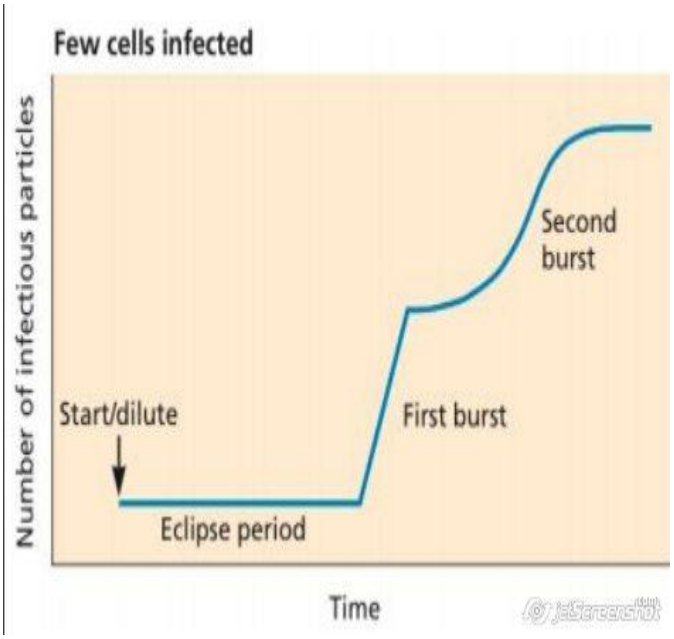
3) **Burst (yield) phase**: this happens when the first virus generated leaves the cell. A high number of viruses are produced (coming out of the cell) through this phase and at that point, the curve goes up until it plateau (all cells are dead and the viruses are released from them).

4) **Burst size**: number of virions released per bacterium.



In single step cycle, all cells are synchronized, which means that all cells are simultaneously receiving the viruses (infected simultaneously) → all host cells go through the same phase of the replicative cycle → they go under the eclipse phase at the same time and synchronized in burst phase → they release all the viruses at the same time .

This is called one step because all the cells are infected ☺

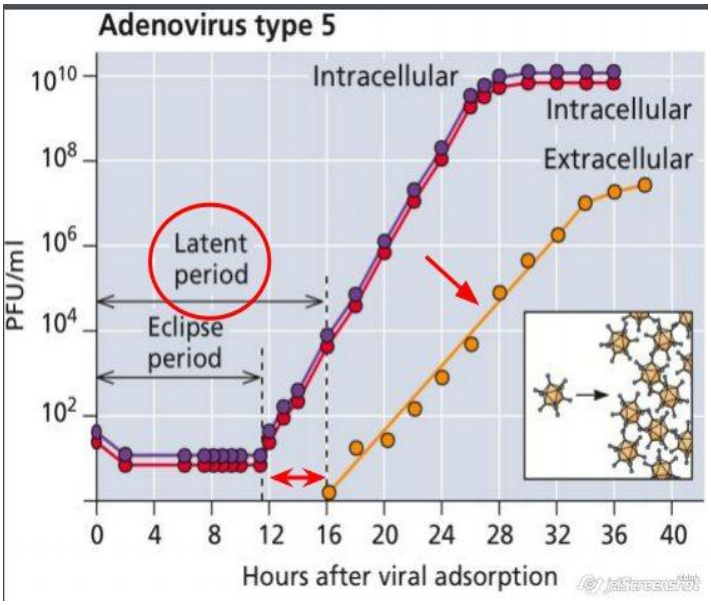


In multi-step growth cycle, only a fraction of the host cells are infected at the inoculation phase.

When a small population of host cells is infected, the viruses components are being synthesized in the eclipse phase and the viruses are leaving the cell outside in the burst phase (first burst phase).

During this first burst phase, the infectious viruses that are produced from this phase will infect other cells that are not infected (healthy), and then they start producing more viruses, causing the second burst phase.

One population is infected and will burst, then the product of that burst infects the second population

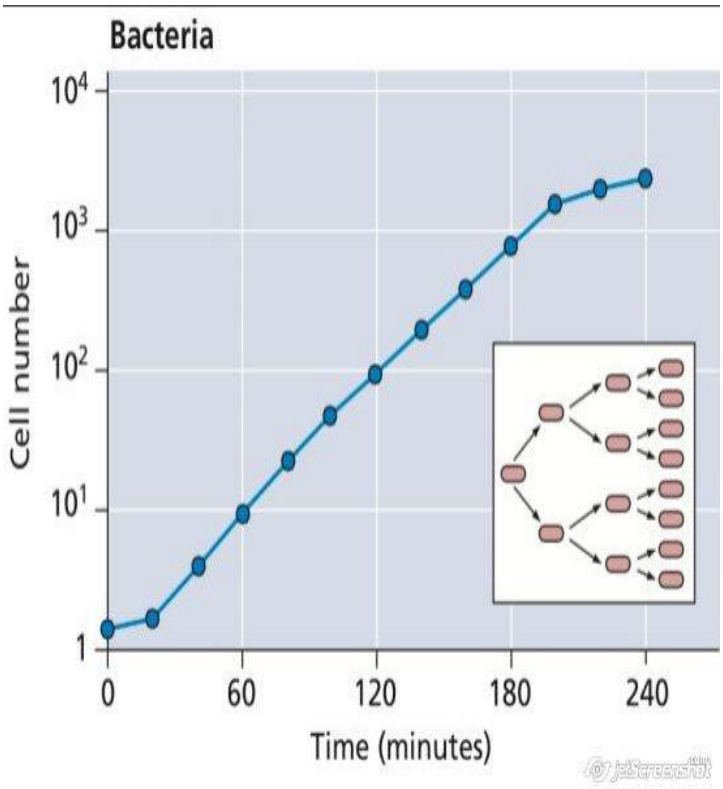


-This curve shows the numbers of extracellular viruses in a sample.

- After the inoculation phase, the eclipse phase of the adenovirus type 5 (where there is no infectious viruses) starts and takes 12 hours in synthesizing viral components, then the burst phase starts.

-The extracellular viruses took 16 hours to appear and this is called the latent period.

-**Latent period**: the period of time where the viruses first appear extracellularly and where there has been no infectivity outside the host cells yet.
There is a gap between the eclipse phase (making an infectious virus in a cell) and latent phase (getting the viruses out into the medium) "lag".
-The reason of this lag is that you are waiting the viral components to assemble together and leave the cell.



(Bacteria VS Viruses):
When a bacterium is put in broth (liquid medium containing proteins and other nutrients for culture of the bacteria), it starts dividing almost immediately, and the growth will be logarithmic because bacteria divide by binary fission. Viruses don't do that,they have to go into the cells to express their genetic information, and make the parts to build the virus particles. That takes time (the eclipse period/ plateau) and its length varies depending on the virus.

Multiplicity of infection (MOI)

-**MOI** ⟶ how many virions (infectious viruses) are added to the cell ⟶ the number of infectious particles added per cell.

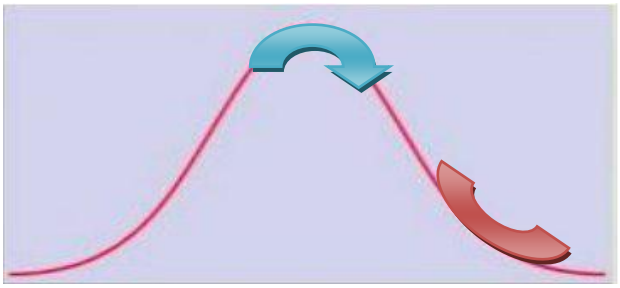
-For example : ten million infectious particles added to million cells⟶ MOI is 10 virions ⟶ not all the cells receive 10 virions.

-There is a difference between adding viruses to the cell and the cells receiving the viruses⟶ adding viruses to the cell doesn't mean that every cell will receive viruses.

-Infection depends on the random collision of virions and cells. When susceptible cells are mixed with viruses , some cells won't be infected, others receive one, two, three or more particles.

-Different cells receive different number of viruses, the number of infectious particles each cell recevies follows a normal curve distribution called a **Poisson Distribution**, its shape follows this equation:

$$P(k) = e^{-m}m^k/k!$$



The region (in the blue color) receiving more infectious viruses than the region (in the red color).

-The equation tells us how many viruses that cells are really receiving.

P(k) = fraction of cells receiving the virus or fraction of cell infected by k virus particles.

K = number of viruses received.

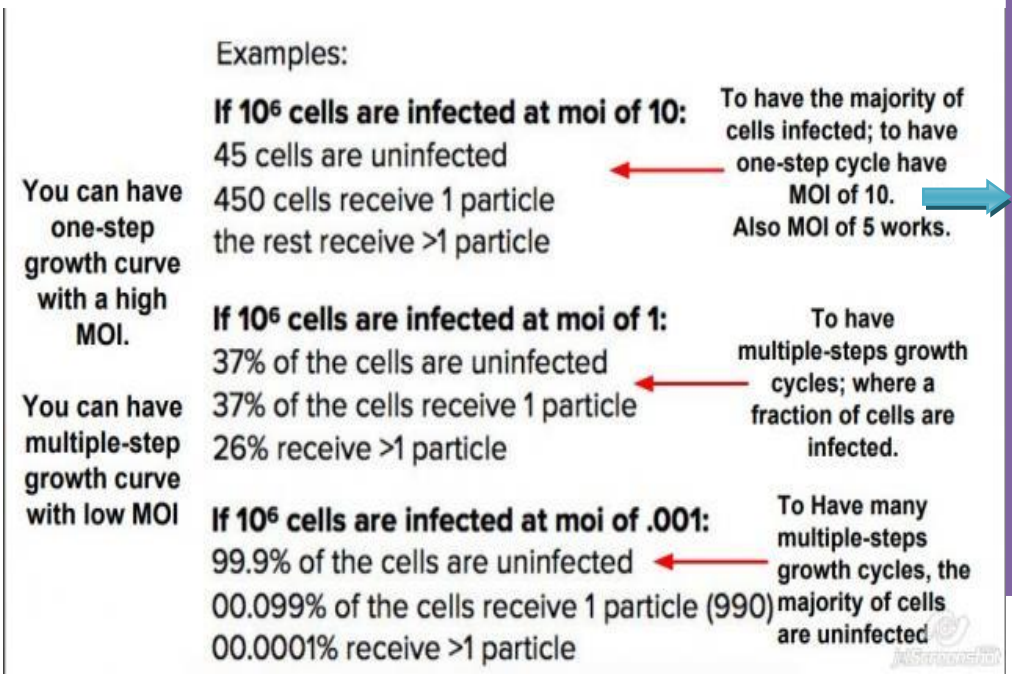
e = natural logarithm

m = MOI

-To know the number of uninfected cells (the number of viruses received by cells is zero)⟶ k=0⟶ uninfected cells = $p(0)= e^{-m}$

-To know the number of cells that received 1 virus ⟶ k=1 ⟶ $P(1) = me^{-m}$

- **The equation is not for memorizing** ☺



Negligible (very small) proportion of cells are uninfected , and most of the cells are infected (with high MOI) ⟶ one-step growth cycle because the cells are synchronized (all cells are infected simultaneosly) ☺

-When **MOI is high** ⟶ MOI of 2 digits (MOI is around 10) ⟶ one-step growth cycle

-When **MOI is low** ⟶ MOI is 1 or less ⟶ multi-step growth cycles.

Physical Measurment of Viral particles

The following methods are for titering or measuring the number of viral particles (infectious+non-infectious):

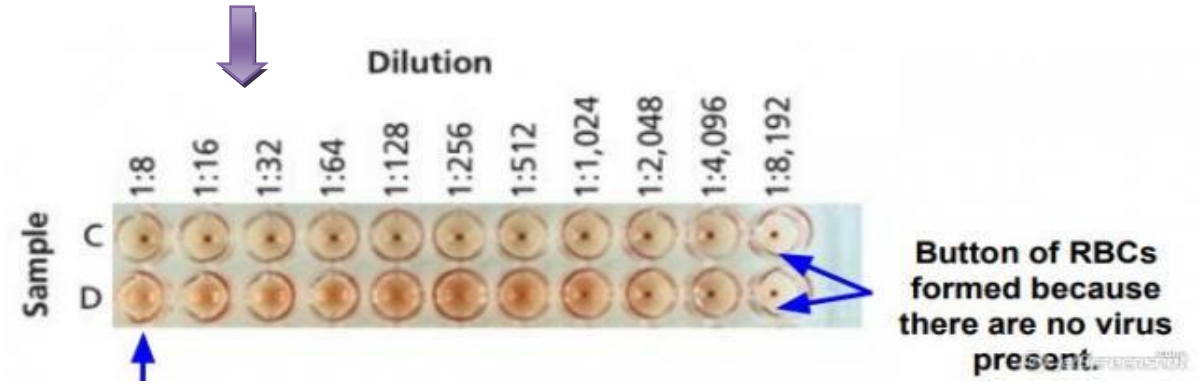
1) **Hemagglutination**: it's very old assay, easy to do, cheap (no reagent, no DNA and no fancy machines) and it's based on the fact that some viruses (like influenza virus) will bind to a sugar (sialic acid) which is the receptor (on the surface of the blood cells) that the virus use to get into the cell.

-This method is done by adding blood (not from the patient) to 96 wells in a plate. We make serial dilutions to the patient's sample (it can be a blood sample or stool sample). After that, we mix the sample dilutions with blood cells in the wells.

-If the virus is present in the patient's sample ⟶ the viruses will stick with the red blood cells ⟶ blood cells will form a lattice (depending on how much viruses are present) and precipitate onto the walls of the wells ⟶ no button will form.

-If there is no virus in the patient's sample ⟶ blood cells stick together and sink to the bottom of the well forming a button.

- The sample dilutions are done in equal increments.



-**Sample C**:control sample (Healthy individual) or just blood mixed with water. Blood buttons are formed in all dilutions → so, there's no virus.

-**Sample D**: there's a virus (because no button is formed in the first dilution) until the dilution of 1:1,042 → at this dilution point → the button is formed → the sample is very diluted (no viruses) → HA titer is 1,042.

-**HA titer (hemagglutination titer)** : is the point at which the sample containing a virus becomes too diluted and shows the first appearance of a button instead of lattice.

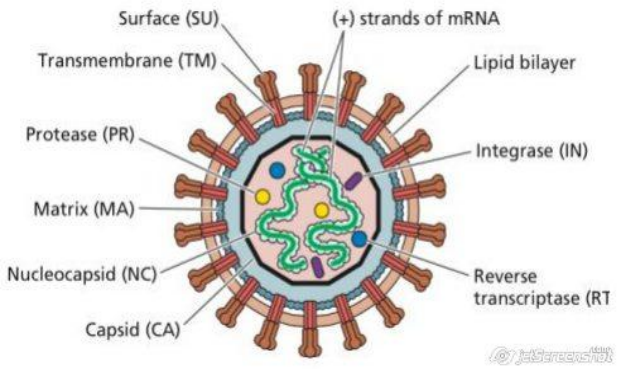
-This method can be used to determine if there is a virus or not in a patient's sample and to compare between two samples of patients with same viral infection, so we can know in which sample the number of viruses is higher.

-Example: Which patient has a higher concentration of viruses than the other?

	Patiet A	Patient B
HA titer	64	**1,042**

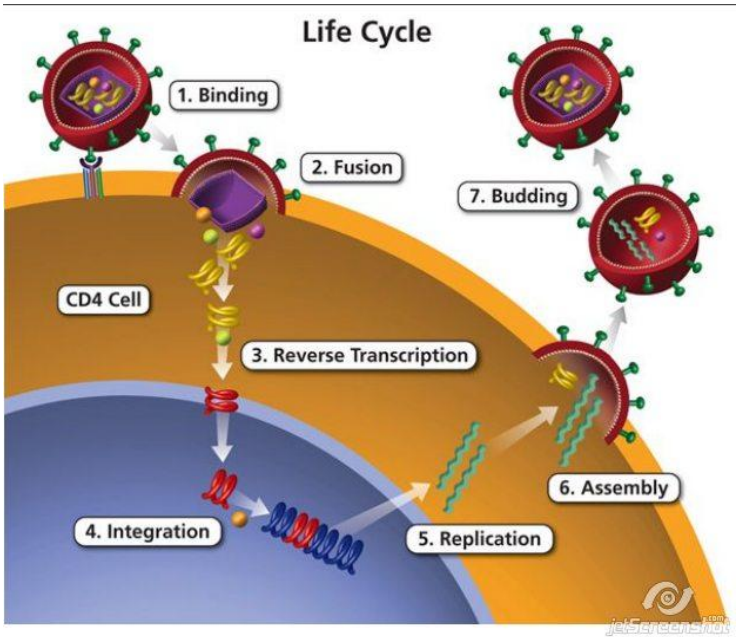
-Answer : Patient B because he has a higher HA titer so more dilutions are needed to start the formation of the button and for the lattice to disappear.

2) Measuring viral enzymatic activity:



-Retroviruses have RNA genome.

- Within the particle there are a variety of enzymes, one of them is the reverse transcriptase enzyme which is used as an example in the measuring the viral enzyme activity.



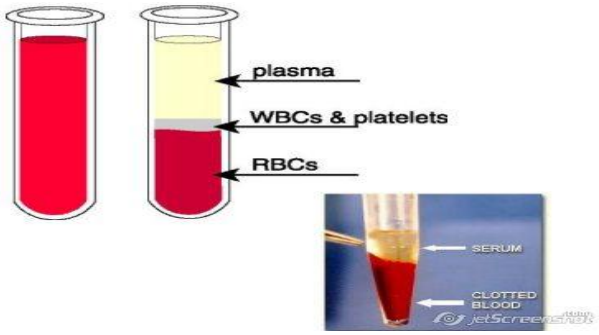
{EXTRA INFO}: The HIV virus binds to the T cell that has CD4 receptor on its surface. It internalizes due to the presence of membrane protein called gp120 on it.
-Then, reverse transcriptase converts the viral RNA to DNA, the virus integrates its genetic material to the host cell DNA, so every time the cell divides, the daughter cells and their chromosomes will have HIV virus.

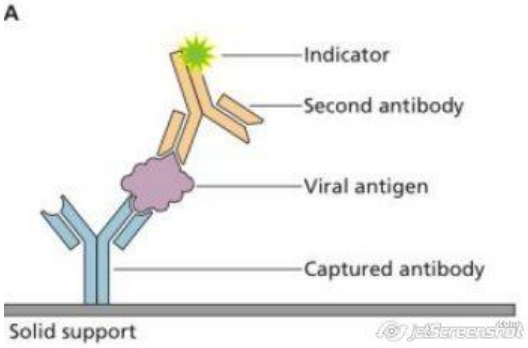
-Measuring viral enzymatic activity: this method is used to determine the number of viruses based on the viral enzymatic activity (the activity of a unique enzyme in a virus).
*We won't get into details, just keep in mind that:
More enzyme activity (reactions) ➡ More enzymes ➡ More viruses

3) Serology:

There are multiple serologic techniques that can be used to measure viral particles, and the most famous one is ELISA.

Enzyme-linked Immunosorbent Assay (ELISA)





-This method uses enzymes, antigens and tags, it's very commonly done, and we can either look for viral protein or antigens or for antibodies in the serum to see if infection happened by a virus.

-When we want to count the viral particles in a patient's sample (to test the viral antigens):

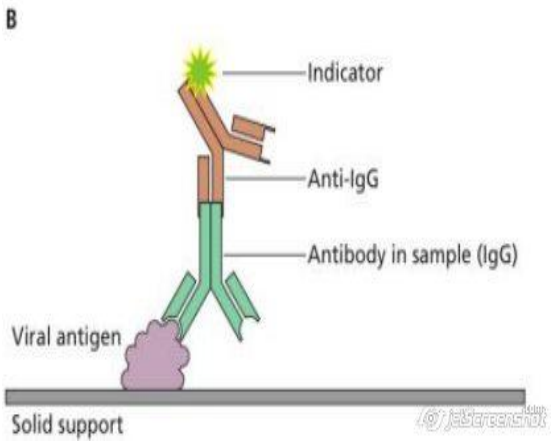
At first, we do blood centrifugation for the patient's blood ⟶ the plasma will be placed at the top of the tube and blood cells (RBCs+WBCs) at the bottom of the tube. Then we remove the clotting factor from the plasma in order to extract the serum. The serum is then incubated in a 96-well plate ⟶ each well has an antibody attached to the solid surface that recognizes specific virus (viral antigen) and binds to it. We add our serum sample to the wells, wash away what we added and the attached viral antigen will remain. We add second antibodies, which bind to different sites on the viral antigens, and wash away again. The second antibodies that are still attached to the viral antigen after washing emit signals (mostly fluorescent).

-We can measure the intensity of signals by using a plate reader that gives us an idea about the presence of the viruses.

-No signals ➡ No antigen ➡ No virus

For better understanding:

https://www.youtube.com/watch?time_continue=1&v=6Ue1Hd3dyaQ



-We can use ELISA to look at the individuals if they have antibodies against a specific virus (to test viral antibodies).

-We use the same method to test viral antibodies but instead of having antibodies attached to the solid surfaces, we attach the viral antigen.

-We add our sample to these wells. If the patient has antibodies against this viral antigen, then binding will occur. After that we add a second antibody that recognizes the first antibody and binds to it.

-We can detect the sample's antibody by using the second antibody because it has an indicator attached to it.

Lateral Flow Assay

-This technique is a serological test based on the principles of ELISA. It's an easy and fast test that can be done at home, without the lab, and a widely spread and well known application is the home pregnancy test.

- It is done in a format that can be read out readily.
-This technique is based on capillary flow that allows moving of the antigens towards the antibodies that are attached to the solid support, if there are antibodies that recognize the antigens from the patient's sample ⟶ these antibodies will bind to the antigens and migrate through the capillary action, and then they will get captured by another set of antibodies.
-When a color suddenly appears (in the test line), this means that the antibody is attached to the antigen and migrate with it.
-If no antigens from the sample are bound to the antibodies, the sample will move alone, and the antibodies won't move (no antigens are attached to the antibodies).

-Why are there two lines?

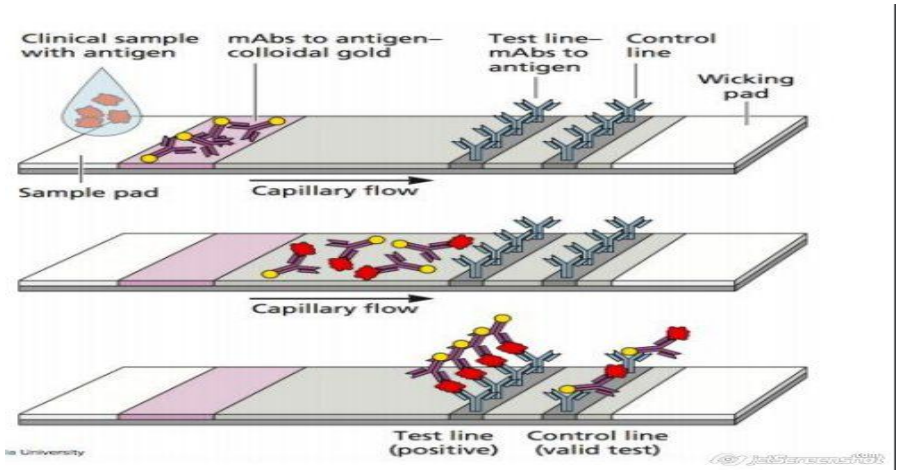
-The initial antibodies can sometimes move due to the power of the capillary flow and not because of the existence of antigens. They can move to the test line but moving to the control line is impossible.

-**Test line**: shows a colored line indicating a positive test.

- **Control line**: to validate the test, to make sure it's not false positive

-In order for the test to be positive:

- Antibodies should pass both lines.



https://www.youtube.com/watch?v=FvIIozN58gw

2) Nucleic Acids Tests:

(To see if there are pieces of viral nucleic acid)

Polymerase Chain Reaction

-PCR (DNA replication in a test tube)

- If we have a sample containing a virus with certain amount but you can't know the amount of DNA that exists because the amount is not high enough to detect it. So if we amplify the initial amount of DNA, we will have an idea about the amount of the virus.

-PCR has been used in many areas:

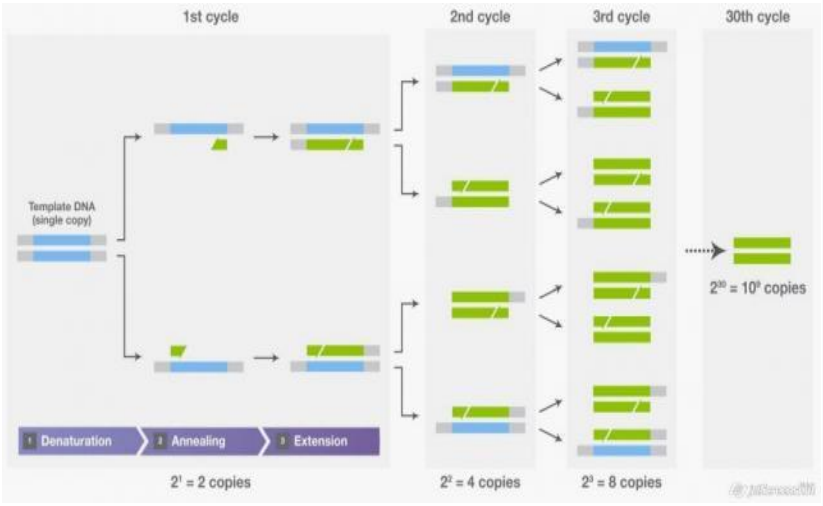
Research -Industry -Diagnosis

- PCR ⟶ replicates a piece of DNA many times over a short period of time using DNA polymerase and primers in the test tube, we use a special type of DNA polymerase that can tolerate temperature, it's extracted from a bacterium that lives in the host spring.

- We choose a region of viral DNA that has several hundred nucleotides ➡ the region of viral DNA is heated in a test tube (Denaturation) ➡ we add primers that are unique for this viral DNA sequence and don't recognize the DNA of the host cell DNA replication takes place by enzymes.

-We repeat these steps until the amount of viral DNA becomes high enough to detect.

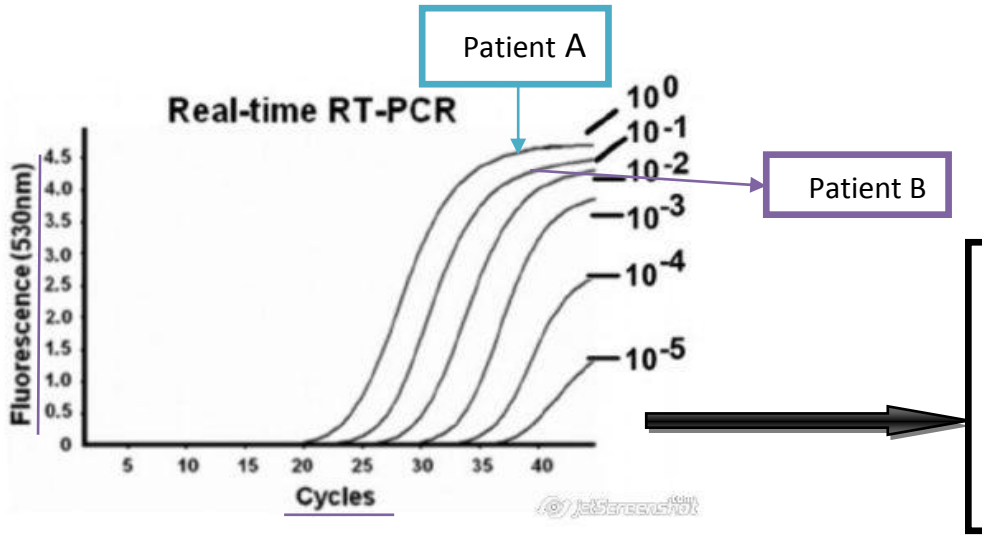
-The final quantity represents how much the initial quantity is. (You just have to do the math) ☺



-One reaction of PCR ➡ (cycle)
-Each cycle doubles the amount of DNA from the previous cycle (an exponential effect).
- Cycle (30) ➡ there is high amount enough to be visualized.
-One template (may have thousand of viruses).

Real-Time PCR

-A new type of PCR was developed to quantify the result (strong quantitative test).
-We do all the steps that are done in the traditional PCR but the nucleotides that the DNA polymerase uses to synthesize the amplified DNA are fluorescent. There is a special device that detects the signals and measures the fluorescence.
-As the reaction goes through many cycles (DNA is being synthesized) ➡ more and more fluorescent nucleotides are added ➡ the sample will become brighter.



Fluorescence curve is used to compare between patients that are infected with the same virus.

-Assume that patient (A) and Patient (B) are infected with the same virus (Hepatitis C), look at the number of cycles, which one of them has more viral particles than the other?

Answer: Patient A.

-For the patient who is infected with more viral particles, his curve will appear faster than the other ➡ patient A is more infected than the patient B because his curve appeared after 20 cycles but the curve of patient B appeared after 25 cycles.

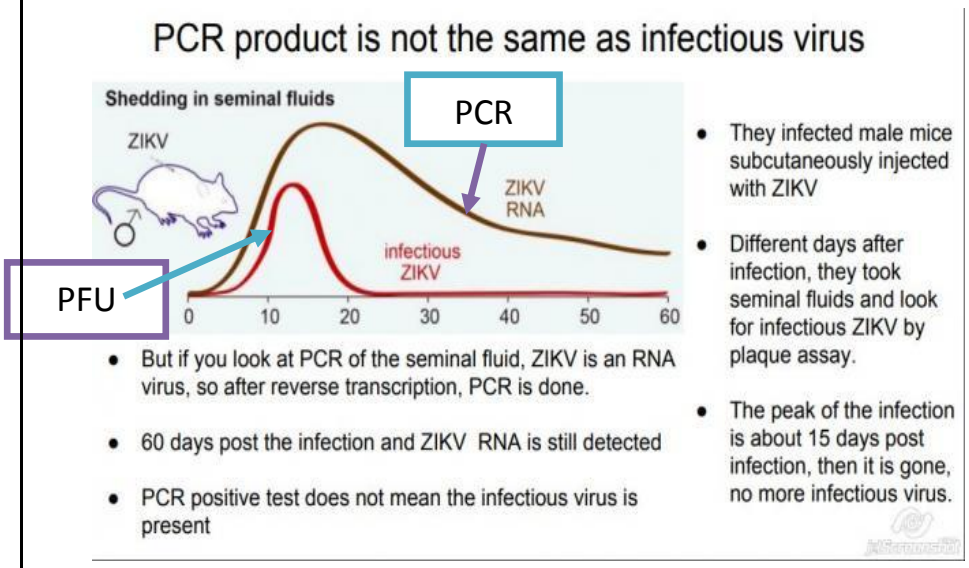
- If there are more templates ➡ more viruses

-PCR product is not the same as infectious virus

- PCR and real-time PCR don't measure the infectivity.

-PFU is the gold standard for measuring the infectious viruses.

*You might someday use PCR in clinical approaches but you always have to keep in mind its limitations.

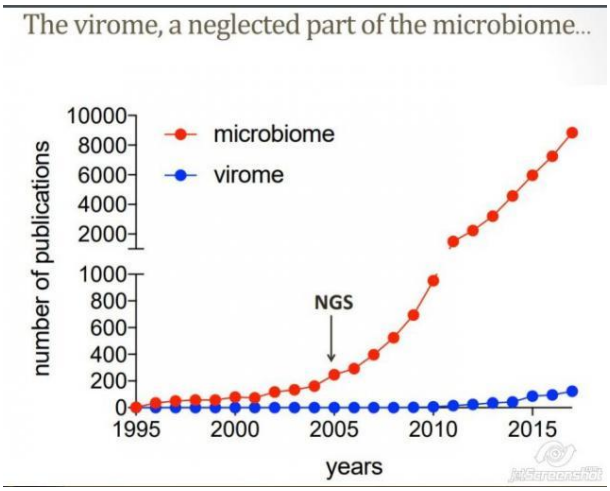


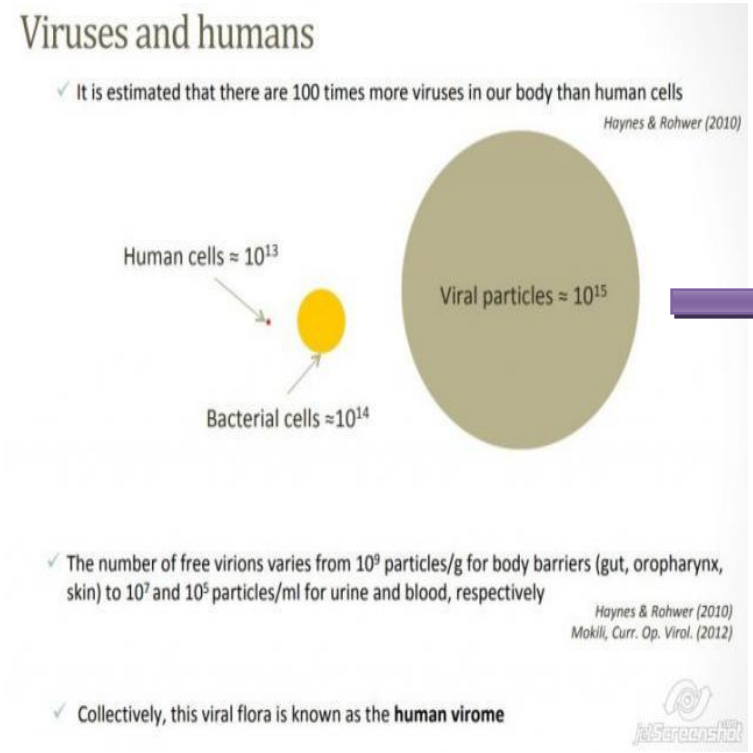
Deep, high-throughput sequencing (NGS)

It's a new technique that can detect many different sequences, can sequence the entire genome of any organism in only 6 weeks, and only costs $1000 making the human genome sequencing relatively cheap (the first human genome sequence took 10 years and cost 3 billion$).

-**NGS** ➡ it's discovering new viruses, our tissues and our organs that what viruses they have.

-Because of this revolution (NGS), the number of publications on the human microbiome has grown exponentially since 2005. The human virome, however, has yet to show an increase in yearly publications.





-There are 10 bacteria and 100 viruses for every human cell in your body even in the none diseased state → not all viruses cause disease.

GOOD LUCK ☺