Lecture 3 Topics:

Particles-to-PFU

Single step and multi step growth cycles

Multiplicity of infection (MOI)

Physical measurements of virus particles

- Hemagglutination
- Electron Microscopy
- Viral enzymes: measuring the viral activity through measuring the viral enzymes.
- Serology
- Nucleic acid: PCR, real-time PCR, deep sequencing

Particles-to-PFU ratio

- Not every virus made in an infected cell is actually infectious.
- Many of them are not infectious even though they look like a normal virus particle.
- Particle-to-PFU ratio is defined by:
  - # of physical particles / # of infectious particles
- To count the number of physical particles, there are 2 ways:
  - looking at them in an electron microscope.
  -using some other assaye.
- After that a plaque assay is done; to count the PFU

Particles-to-PFU ratio

- It is not 1:1 ratio for most viruses
- Infectious particles maybe are rare in a population of viruses
- One-hit kinetics curve means that a single particle can initiate infection, but NOT all viruses are successful in doing that
- That's because:
  - they are damaged
  -they have mutations
  -the infectious cycle is complicated.
- Whatever the reason is, you can never know (depending on the virus), if the physical particles are actually having the effect that you are looking at

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

This number means that for every plaque or infectious virus particle (virion) in this preparation: there are 10,000 that are not infectious

This means that for one infectious unite there are 1-2 not infectious viruses.

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Particle is infectious and non infectious.
PFU is infectious (virion).

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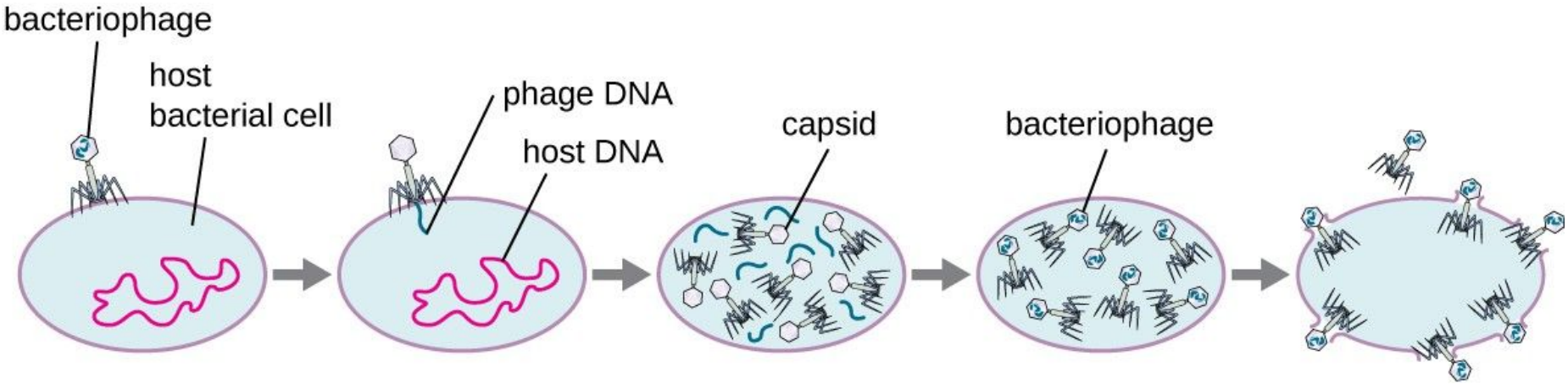
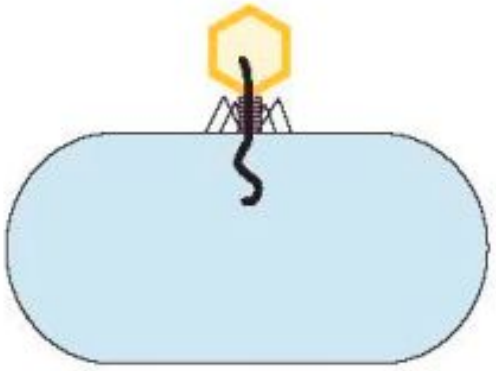
Studying the virus infectious cycle

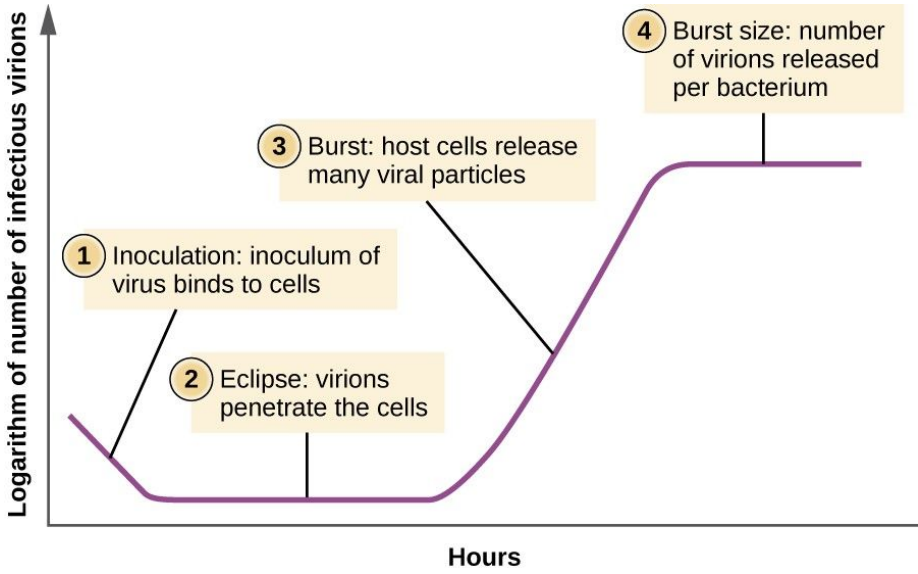


1. **Attachment** The phage attaches to the surface of the host.
2. **Penetration** The viral DNA enters the host cell.
3. **Biosynthesis** Phage DNA replicates and phage proteins are made.
4. **Maturation** New phage particles are assembled.
5. **Lysis** The cell lyses, releasing the newly made phages.

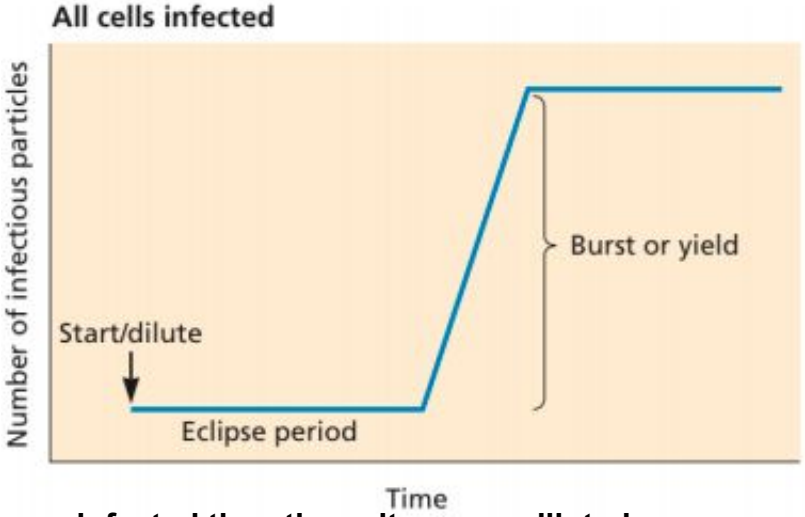
One step growth cycle

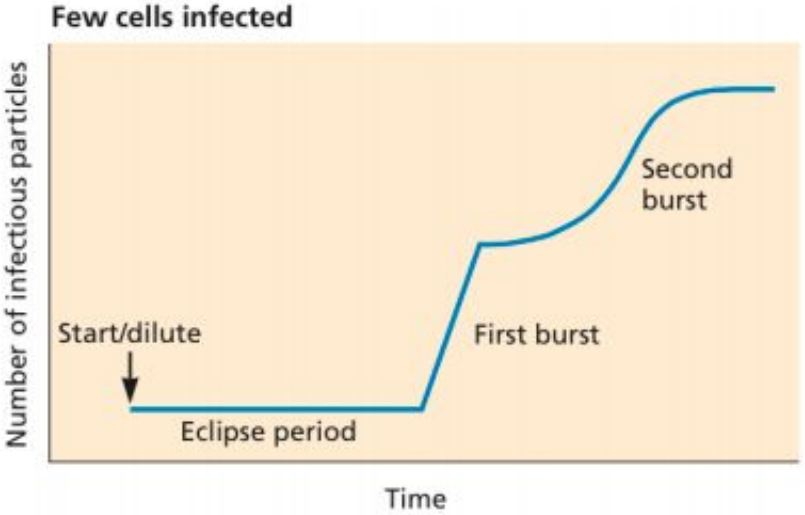


- It was first developed at 1939 by Emory Ellis and Delbruck while working on bacteriophages.
- Take the virus preparation and adsorb it
- Then the culture is diluted, so no more infection occur.
- Then the culture is synchronized (all the cells in the culture are in the same growth stage)
-
- Samples are taken at different times after infection.
- Measure virus by plaque assay. That's one-step growth cycle



Single and multi-step growth cycles





Cells are infected then the culture was diluted so no more infection occurred, so it it is synchronized.
Then we are looking at infectious viruses produced over time.

All cells are infected and release viruses at the same time

Fewer cells are infected for example 10% of the culture.

Single and multi-step growth cycles

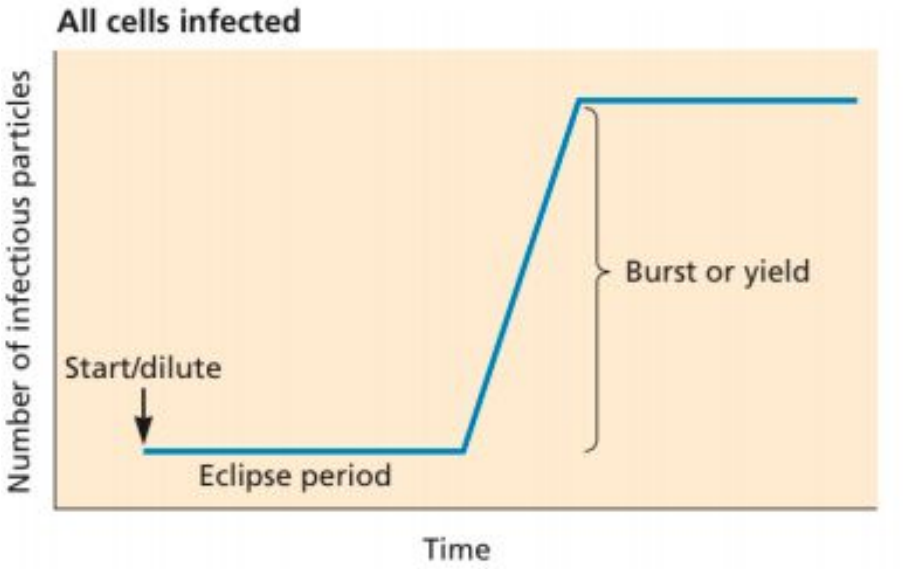
- Eclipse period: nothing seems to happen in term of the number of the infectious particle, but in the cell the genome came out of the virus particle and mRNAs and proteins are being produced.

- Eventually virus particles will be assembled and when those first particles are made, then we see viruses coming out of the cell.

- At that point the curve goes up, until it plateaus when all the cells are dead.

- That phase is called the burst or the yield

Single and multi-step growth cycles



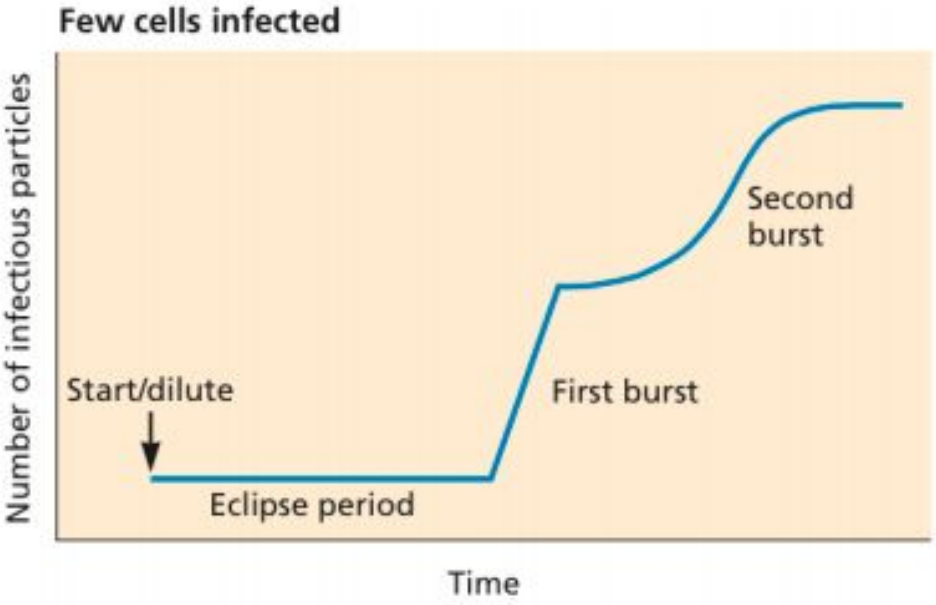
The two key parts here are:

1- **Eclipse period**: apparently no infectivity is being generated, but in the cell lots of synthesis is going on.

2- **The Burst or yield**: this happen when the first viruses generated leave the cell.

This is called one step because all cells are infected, they go through the same phase of the replicative cycle and they all release viruses at the same time

Single and multi-step growth cycles



Here only a fraction of the cells are infected.

At first in those infected cells synthesis of viruses will occur.(**Eclipse period**)

Then those infected cells will release viruses (**thats the first burst**)

That virus will go out and infect neighboring cells, (**then the second burst will happen**.)

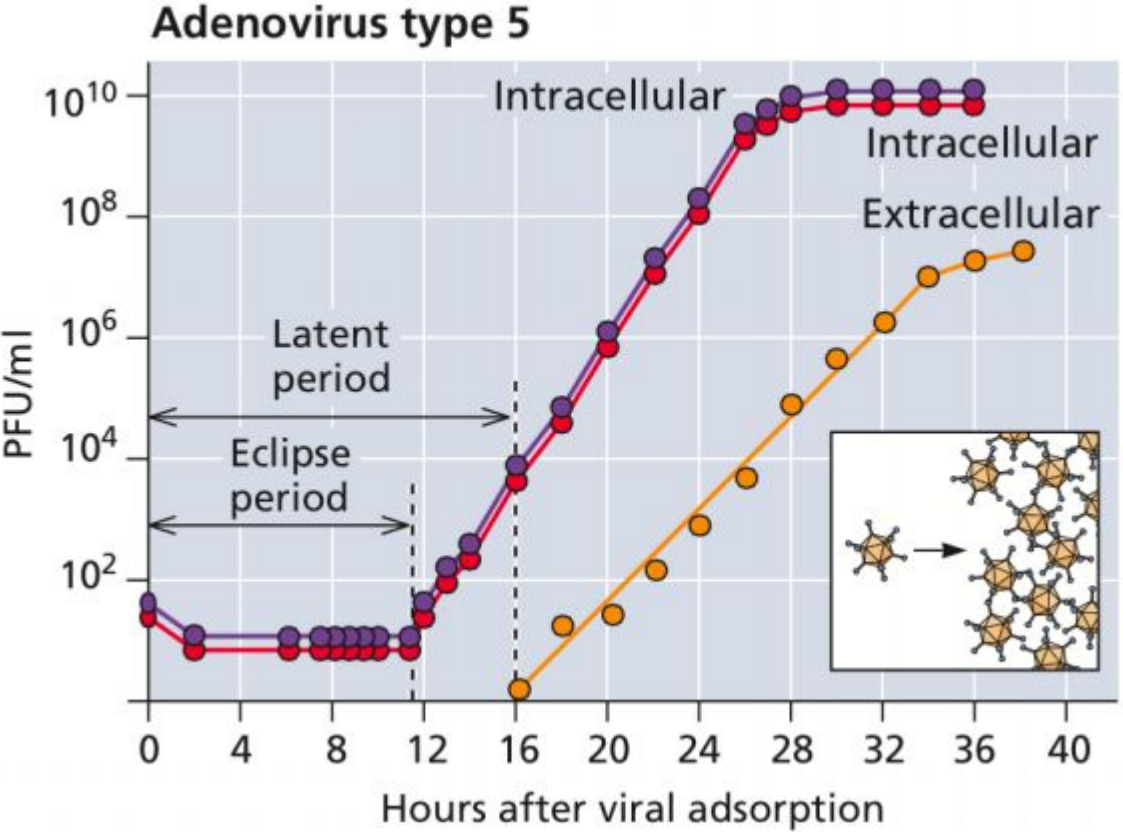
Single and multi-step growth cycles

- Depending on how much virus initially added into the culture there could be

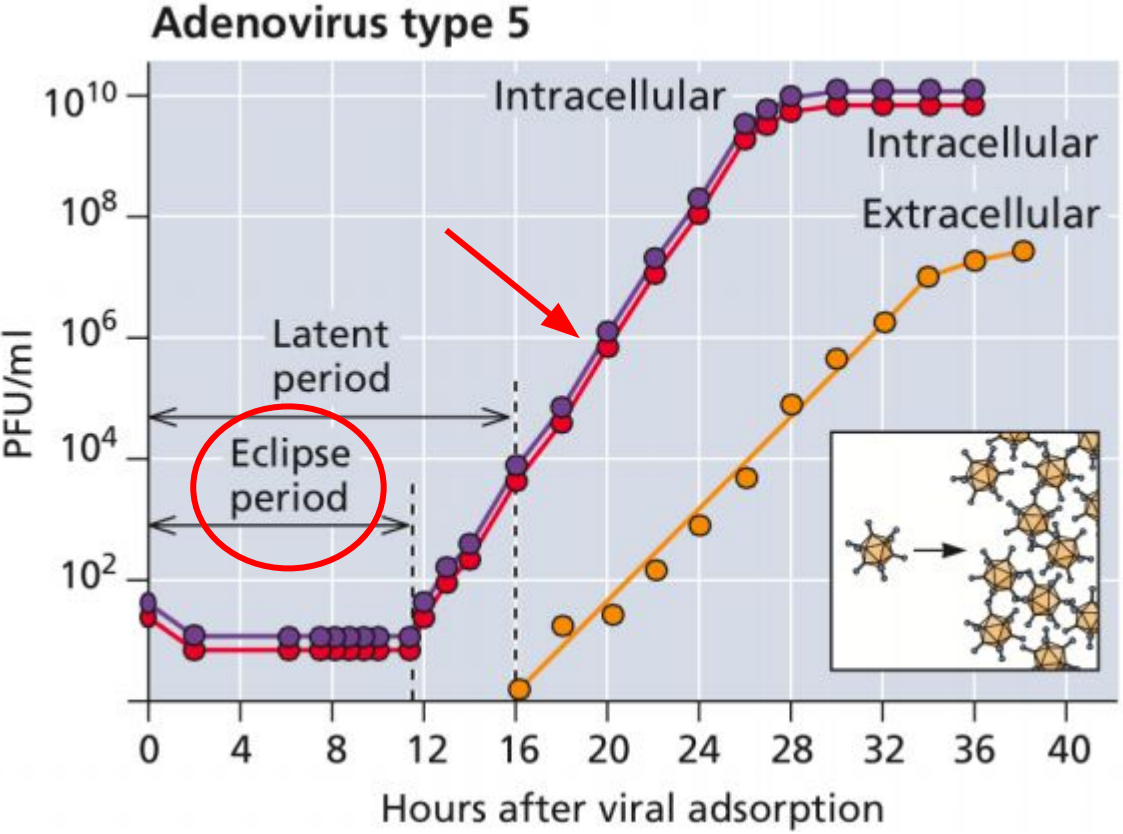
One step growth, where all the cells infected

OR

Multi-cycle growth where only a fraction of the cells are infected at any time.

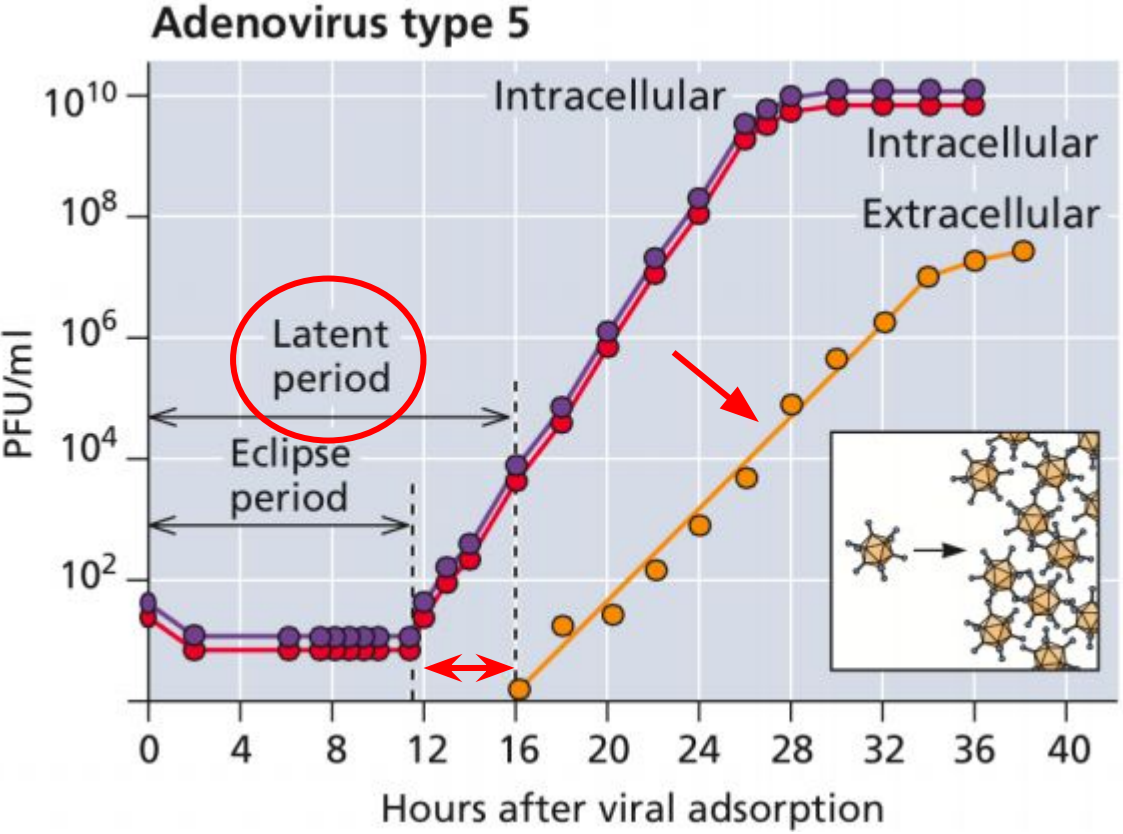


A curve that shows one-step growth, cells are infected, the culture is diluted to prevent more infections.



Eclipse and burst periods can be determined.

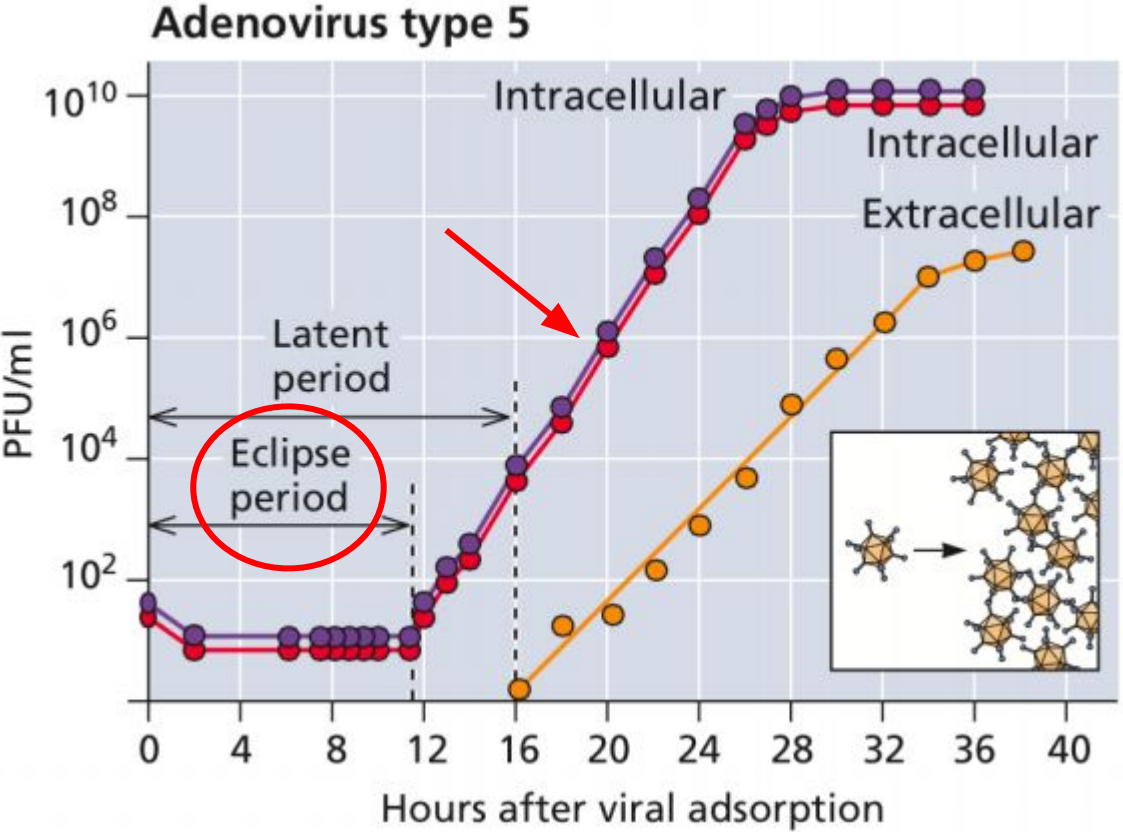
We are actually looking at the intracellular virus particle production, so the cells are broken open at each point time and the infectious viruses are measured.



If we take this one step further and look at the viruses in the medium (extracellular virus), a lag between the time when the first infectious particles can be detected in the infected cells and when they are detected in the medium.
That comprises the latent period.

Eclipse: can't find infectivity in infected cells

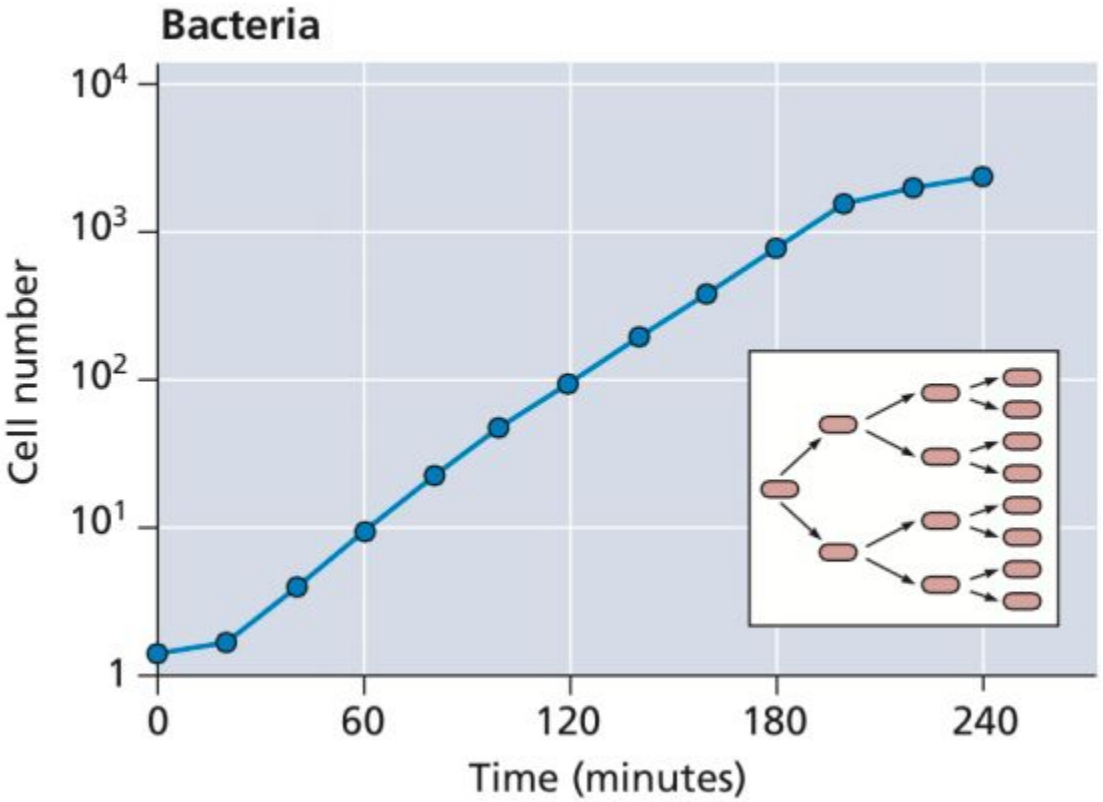
Latent: is when no infectivity can be observed outside the cell in the culture medium



Eclipse period: no infectious virus in the infected cells

Latent period: no infectivity outside the cells.

this means that there is a **lag between making an infectious virus in a cell and its getting out into the medium**.

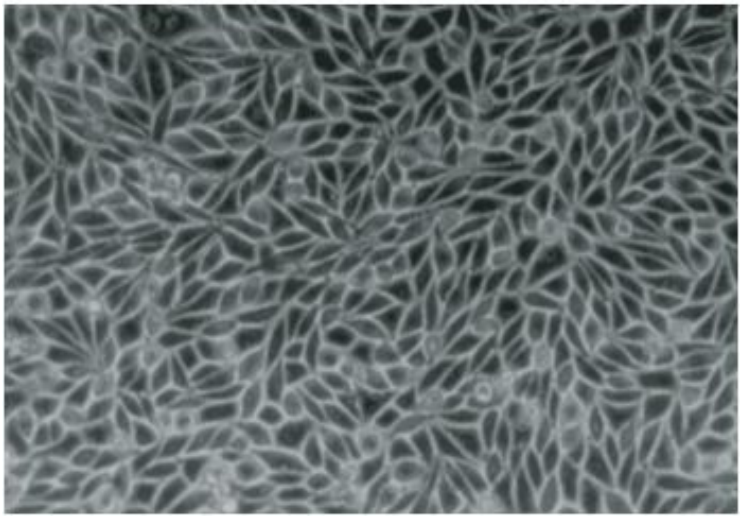


In contrast with bacteria. When a bacterium is put in a broth it begins to divide almost immediately, and the growth will be logarithmic because bacteria divide by binary fission.

Viruses do not do that, they have to go unto the cell to express their genetic information, make the parts to build the virus particles.
That takes time (the eclipse period) and its length varies depending on the virus

Synchronous infection - key to one-step growth cycle

- To achieve one-step growth cycle.
- How do we know that we are infecting all the cells in the one-step cycle?
- It is not a hit or miss.
- We have to know how how much virus we are putting on.



Multiplicity of infection (MOI)

- We have to know how much viruses we are adding on the cells, because we have to get every cell infected.

- The key to understand that multiplicity of infection (MOI); the number of infectious particles ADDED per cell.

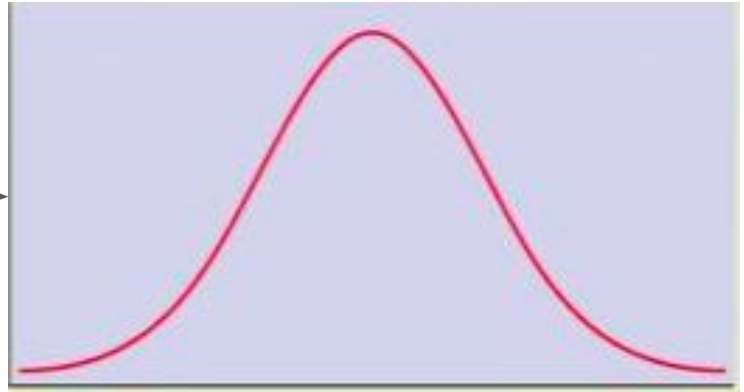
- It is not the same as what each cell receives. It is the number added.

- For example, 10^7 infectious virus particles to a million cells the: MOI is 10 virion, that is not what each cell receives.

MOI

- What each cell actually receives is a distribution which is based on Poisson distribution
- Infection depends on the random collision of virions and cells
- When susceptible cells are mixed with virus, some cells are uninfected, some receive one, two, three or more particles

Poisson distribution, some will get →



MOI

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

P(k): fraction of cells infected by k virus particles

m: MOI

To know the number of the uninfected cells: put k= 0; Uninfected cell: $p(0)= e^{-m}$

To know the number of the cells that receives 1 viral particle: put k=1; $P(1) = me^{-m}$

Cells multiply infected are represented with this formula; $P(>1) = 1-e^{-m}(m+1)$
That is obtained by subtracting form 1
1 is the sum of all probabilities for any value of k, the probabilities P(0) and P(1).
That's how to get P greater than 1

You can have one-step growth curve with a high MOI.

You can have multiple-step growth curve with low MOI

Examples:

If 10^6 cells are infected at moi of 10:
45 cells are uninfected
450 cells receive 1 particle
the rest receive >1 particle

If 10^6 cells are infected at moi of 1:
37% of the cells are uninfected
37% of the cells receive 1 particle
26% receive >1 particle

If 10^6 cells are infected at moi of .001:
99.9% of the cells are uninfected
00.099% of the cells receive 1 particle (990)
00.0001% receive >1 particle

Examples:

If 10^6 cells are infected at moi of 10:
45 cells are uninfected
450 cells receive 1 particle
the rest receive >1 particle

To have the majority of cells infected; to have one-step cycle have MOI of 10.
Also MOI of 5 works.

You can have one-step growth curve with a high MOI.

If 10^6 cells are infected at moi of 1:
37% of the cells are uninfected
37% of the cells receive 1 particle
26% receive >1 particle

To have multiple-steps growth cycles; where a fraction of cells are infected.

You can have multiple-step growth curve with low MOI

If 10^6 cells are infected at moi of .001:
99.9% of the cells are uninfected
00.099% of the cells receive 1 particle (990)
00.0001% receive >1 particle

To Have many multiple-steps growth cycles, the majority of cells are uninfected

Physical measurements of virus particles

- Hemagglutination
- Electron Microscopy
- Viral enzymes: measuring the viral activity through measuring the viral enzymes.
- Serology
- Nucleic acid

Physical measurements of virus particles

- Hemagglutination
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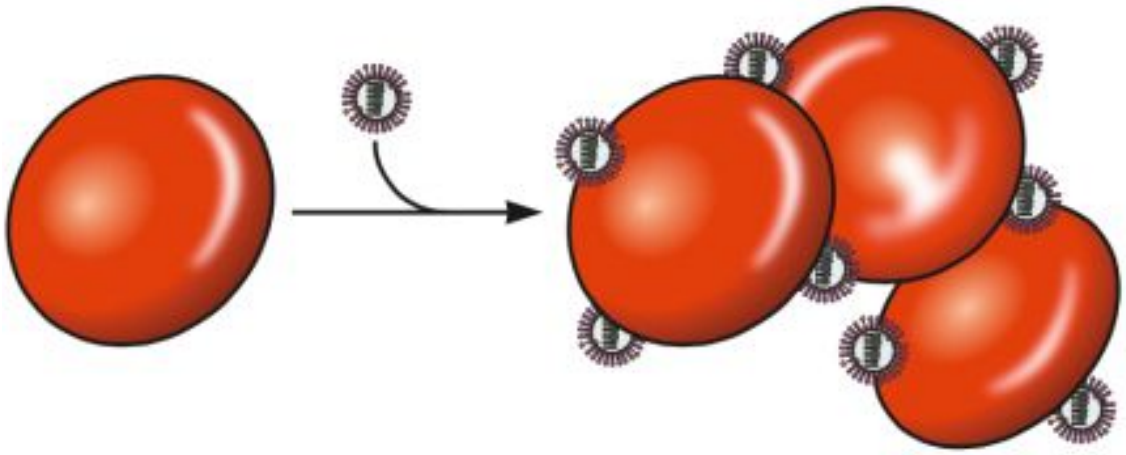
These methods measures virus particles, not telling about infectivity.

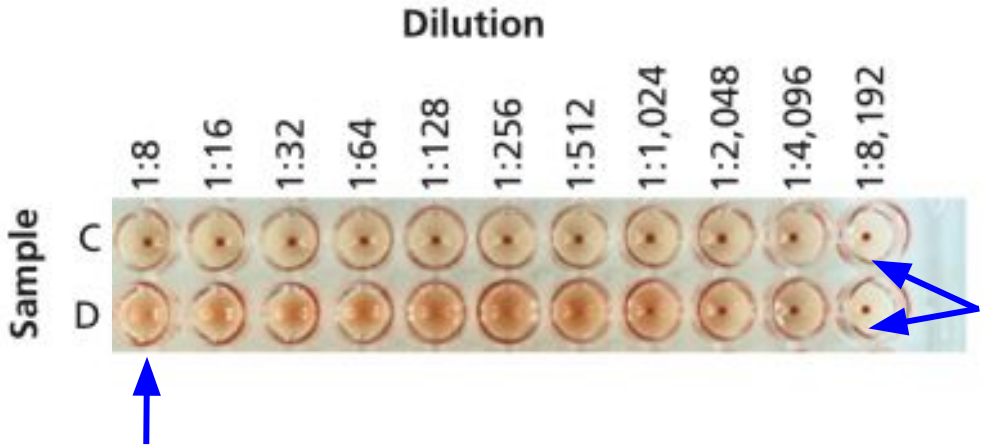
Hemagglutination

- It is a very old assay, it is based on fact the some viruses (like influenza virus) will bind to a sugar (silac acid) which is the receptor the viruses use to get into the cell

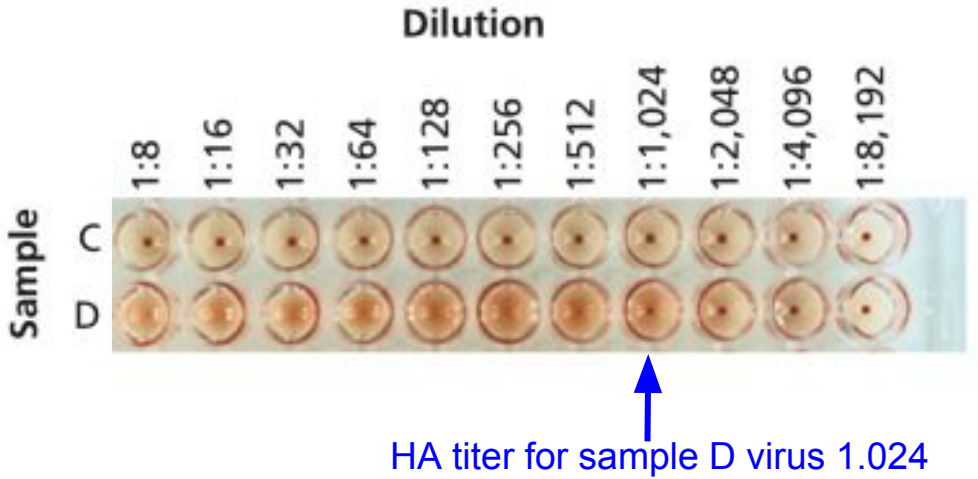
- This receptor is on the surface of red blood cells

- So, if RBCs are taken then influenza virus is added, this virus will stick the RBC and them other RBCs will stick to them and eventually a lattice form, depending on how many virus particles are present.
- If there are not enough viruses the RBCs won't stick together but if there are many they will.





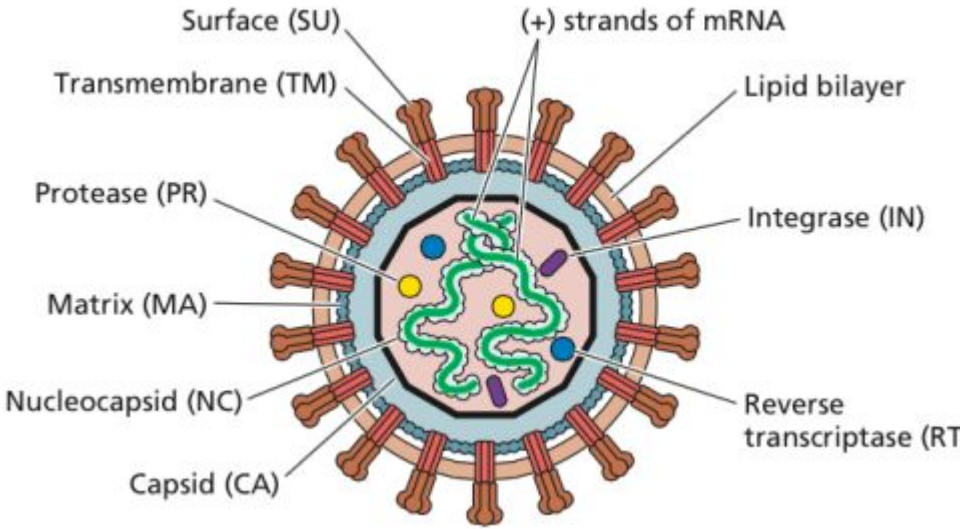
- This is an example of this assay.
- In each well a dilution of virus (1:8 -1:8192), these dilutions are mixed with the same amount of RBCs.
- After about 30 minutes, the RBCs will tumble to the bottom of a well making a little button if there is no virus present to prevent the formation of this button.
- If there is a virus and a lattice coates the sides of the well, there is no button.
- When RBCs starts to tumble at the bottom of the well means that dilution is the end point for this assay.

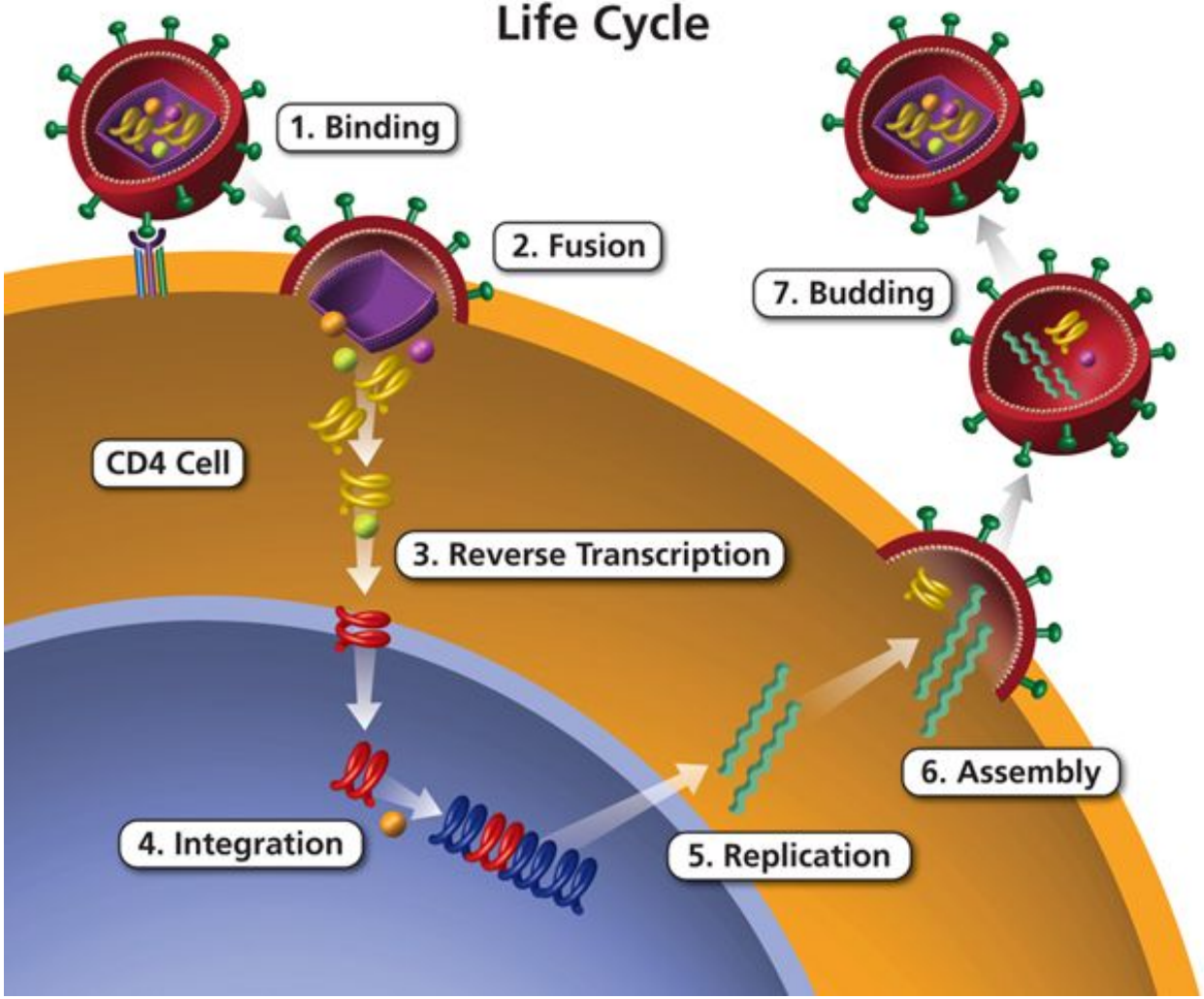


- A quick assay to have an idea before doing cell culture assay

Measurement of viral enzyme activity

- Many enzymes have enzymes in the particle, one of these viruses is retroviruses.
- 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.

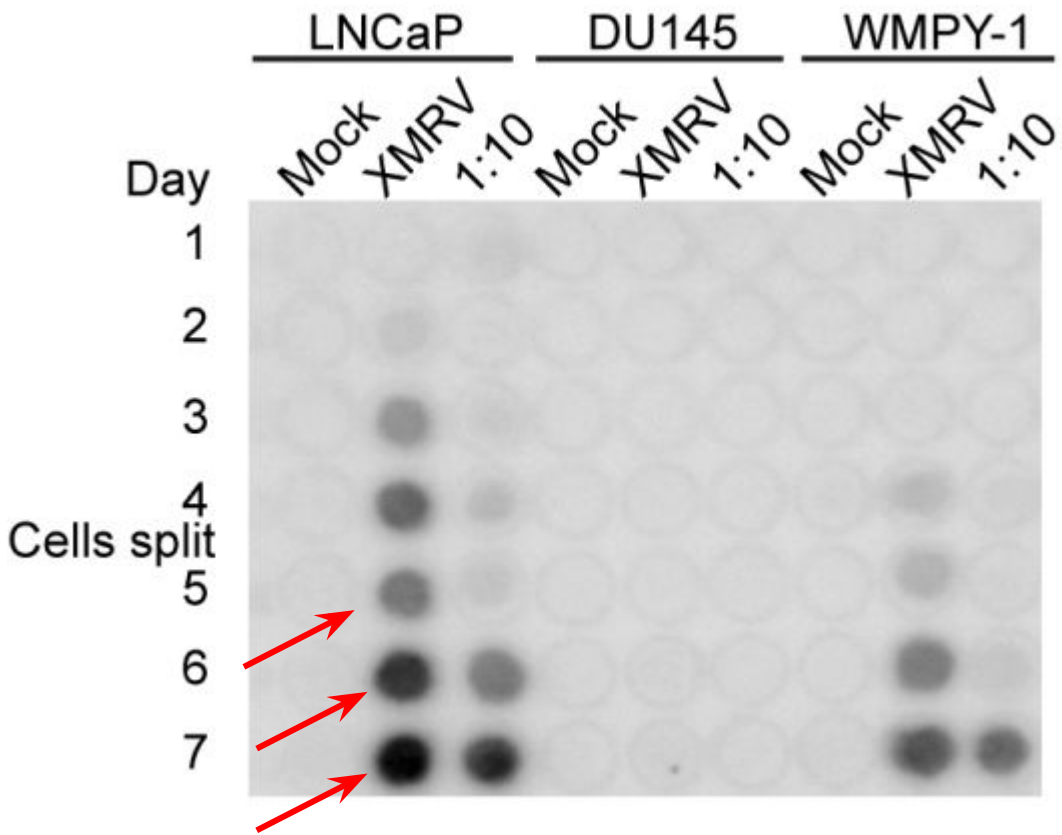



Life Cycle
1. Binding
2. Fusion
7. Budding
CD4 Cell
3. Reverse Transcription
6. Assembly
4. Integration
5. Replication

Measurement of viral enzyme activity

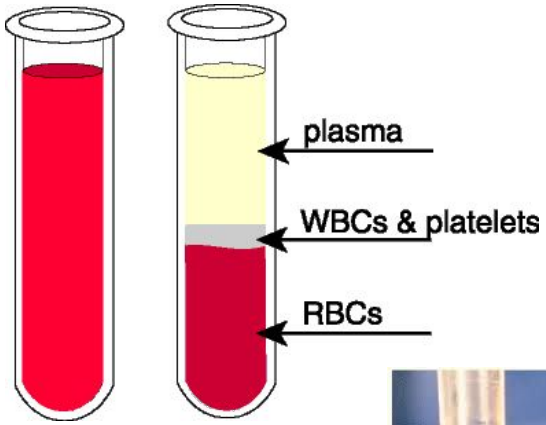
- reverse transcription takes the RNA genome of the virus and make a DNA copy of it
- This enzyme activity can be measured readily, and give an idea about how many virus particles are present
- There is an assay for reverse transcriptase
- A solution with our virus in it -> add to it radioactive substrate the virus needs -> and add a little detergent to increase the permeabilize of the virus
- The substrate will get into the particle, the enzyme will copy it we provide a primer and a template
- and we can measure the amount of radioactivity incorporated

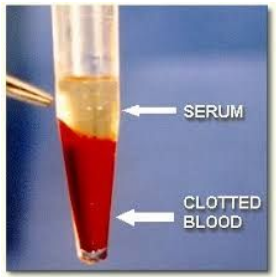
- The Dotes where the materials been filtered through a filter paper and exposed to x-ray film.
- The dots are a positive reaction for the enzyme for this particular virus.
- A dilution can be done to quantify the assay.
- Now this assay is done without radioactivity but with the same principale



Enzyme-linked Immunosorbent Assay (ELISA): detecting viral antigens or antibodies

- It is very commonly done, we can either look for viral protein or antigens or we can look for antibodies in the serum to see if infection happened with a virus.
- Here we are looking for a viral antigen.
- Serum is taken to know if the patient is infected with a particular virus.



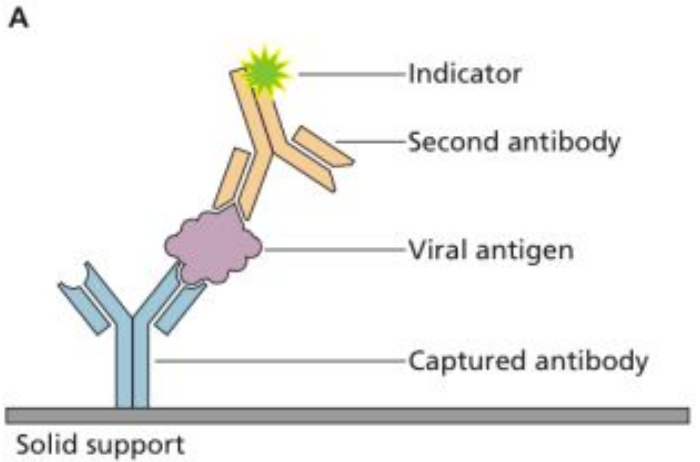


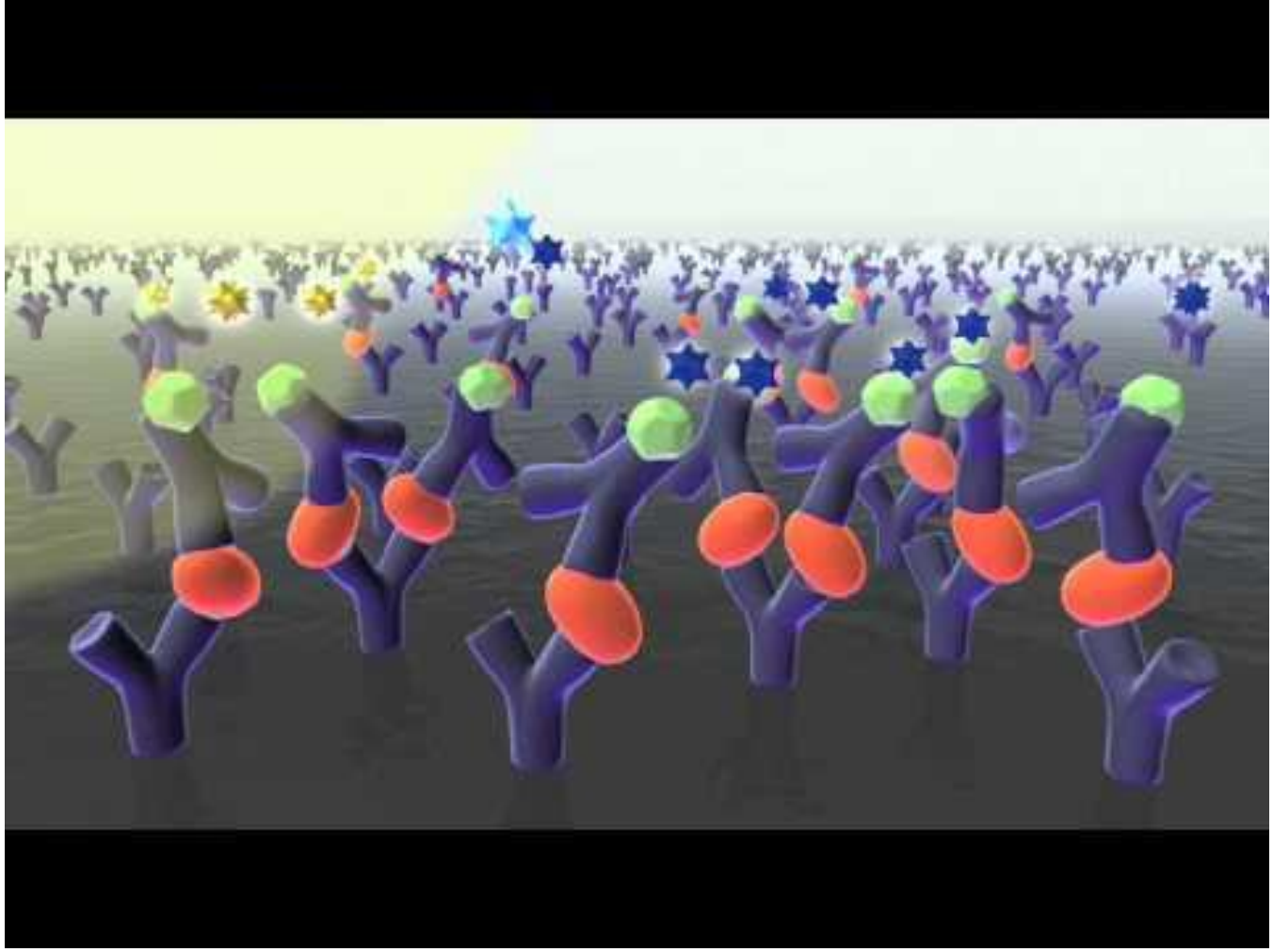
Serum = Plasma – Clotting Factors

Enzyme-linked Immunosorbent Assay (ELISA): detecting viral antigens or antibodies

Viral Antigens

- The sample is incubated with a capture antibody attached to a solid support.
- If the viral protein is present, the antigen will bind to the 1st antibody.
- A 2nd antibody is added to the viral protein, and it has an indicator which can be measured, an enzyme or a light emitting source.





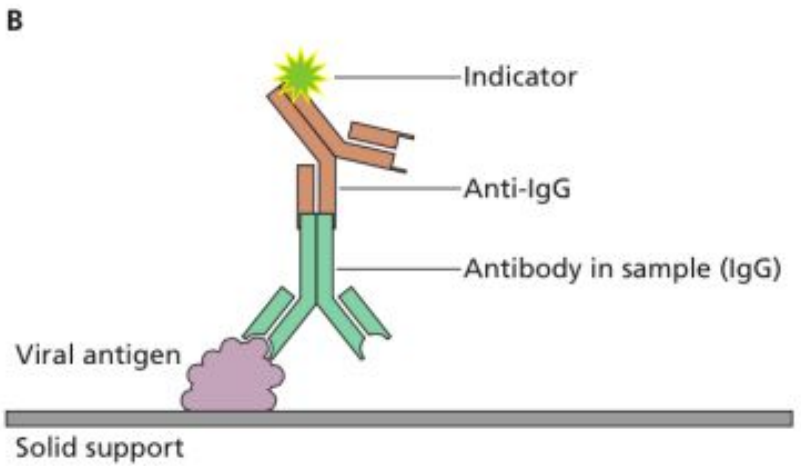
ELISA

Lateral Flow Assay

ELISA

Viral Antibodies

- If there are antigens in the sample, a positive signal will appear.
- It is the same idea with looking for antibodies.
- In this case we attache viral antigen to the solid support.
- Flood the solid support with the sample.
- If the sample has antibodies in it binding Will occur and then we can detect that Antibody with a 2nd that has an indicator.

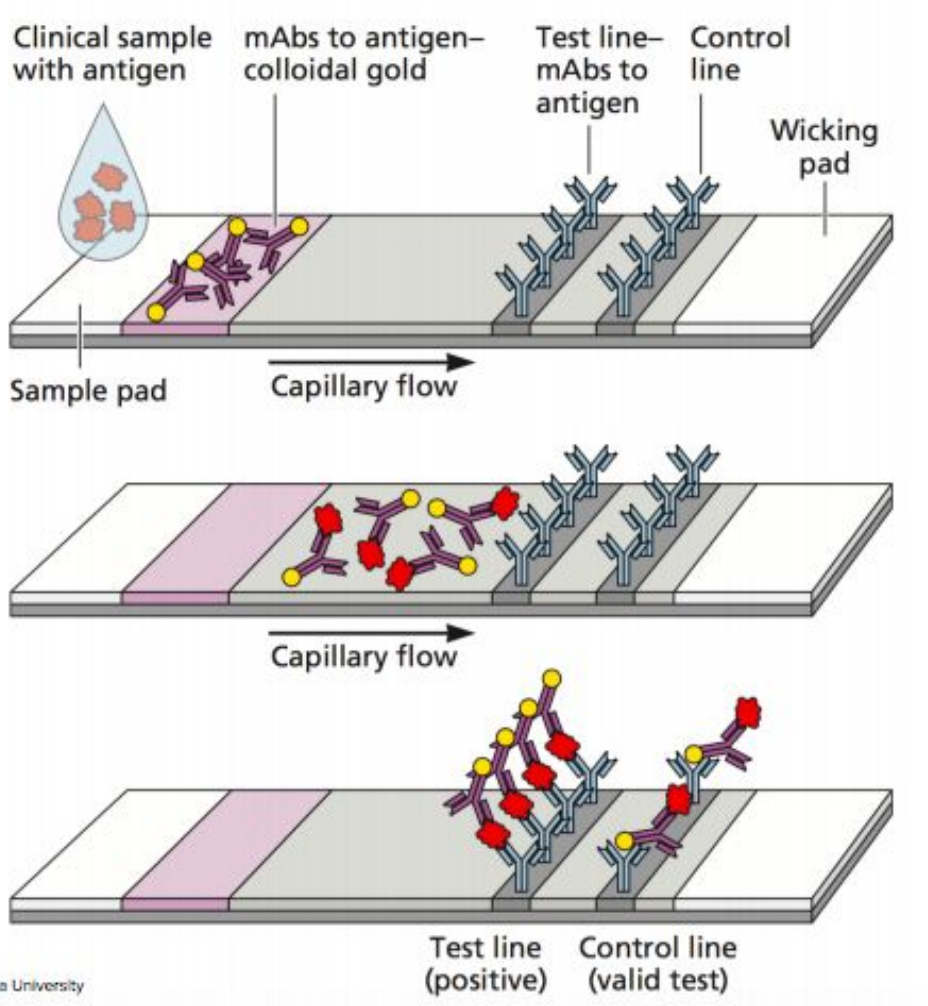


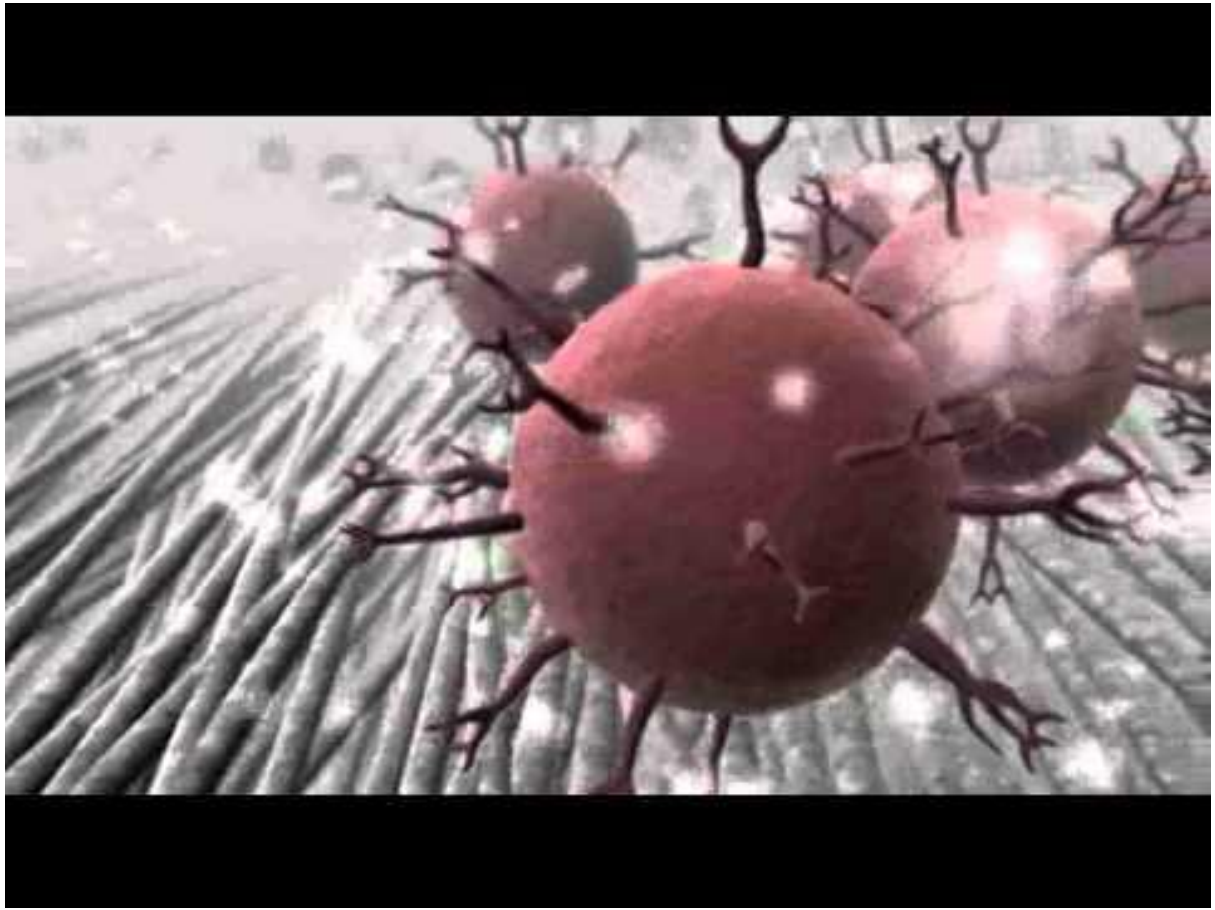
ELISA

- This can be used both in research and in clinical applications.
- Many of these have been developed into rapid assays which can be done in physician offices.
- One of these forms is lateral flow assay.

Lateral flow assay

- It has the same principle as ELISA.
- But it is done in a format that can be read out readily.





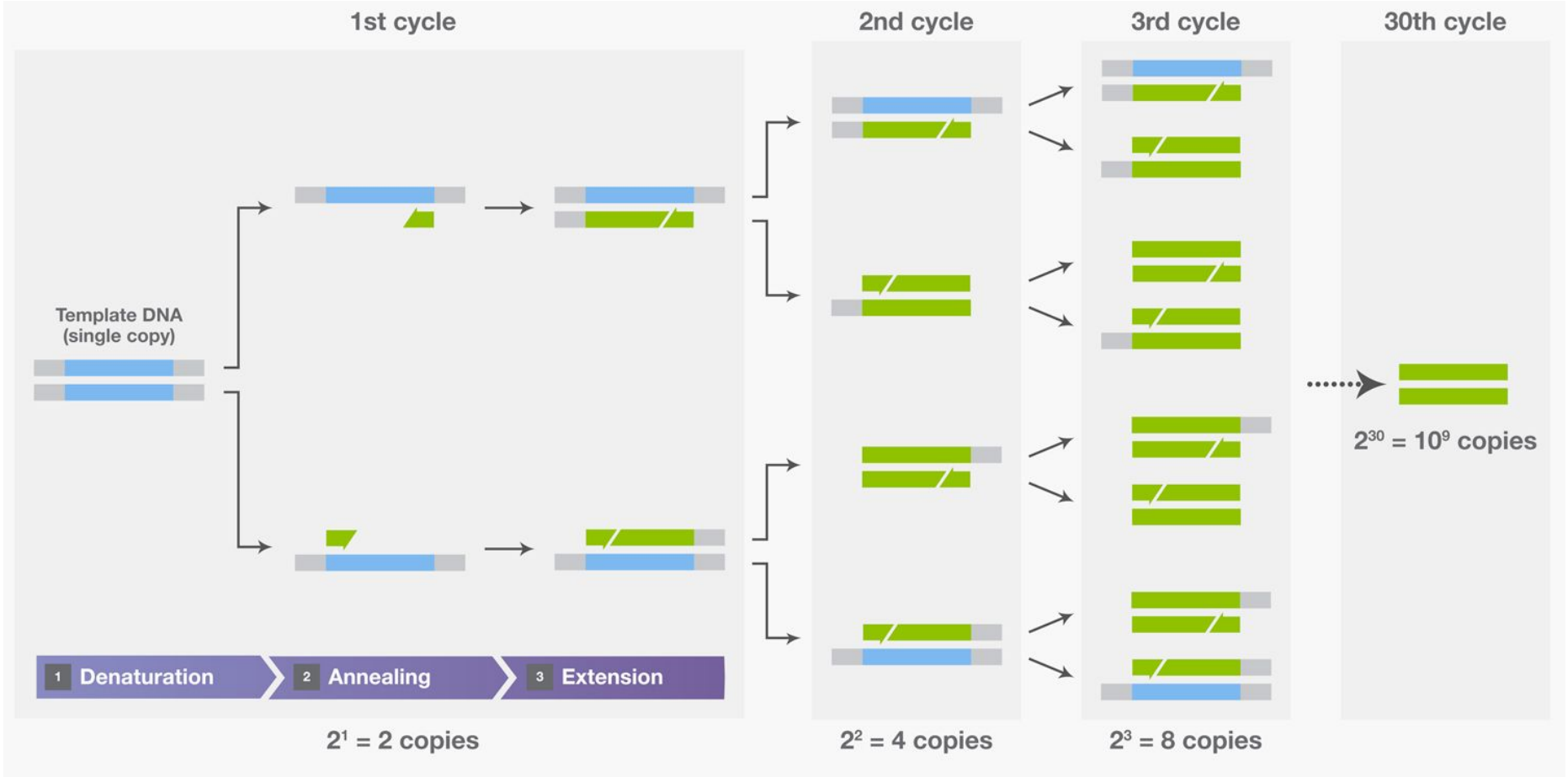
Lateral Flow Assay

Polymerase chain reaction (PCR)

- PCR has been used in many areas:
  - Research
  -Industry
  -Diagnosis

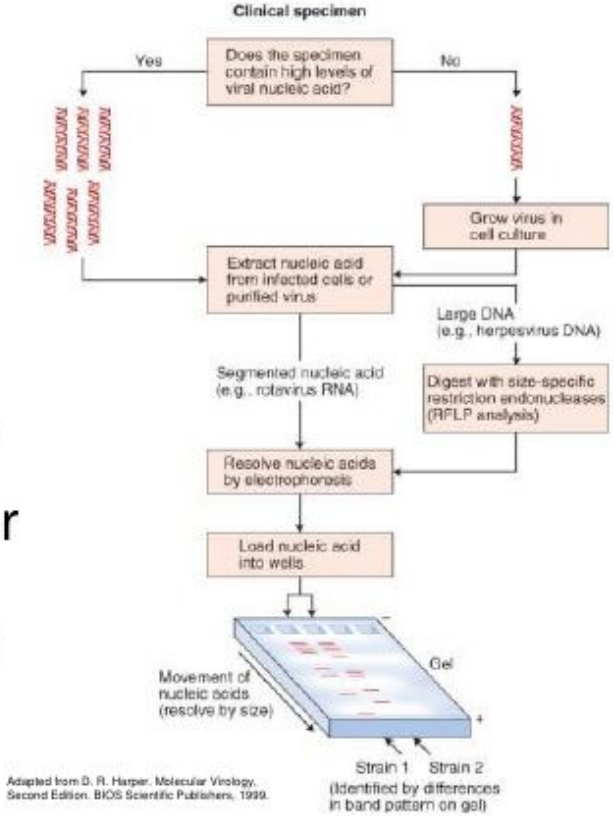
- PCR depends on the use of thermal stable DNA polymerase which came from a bacteria that lives in hot springs.

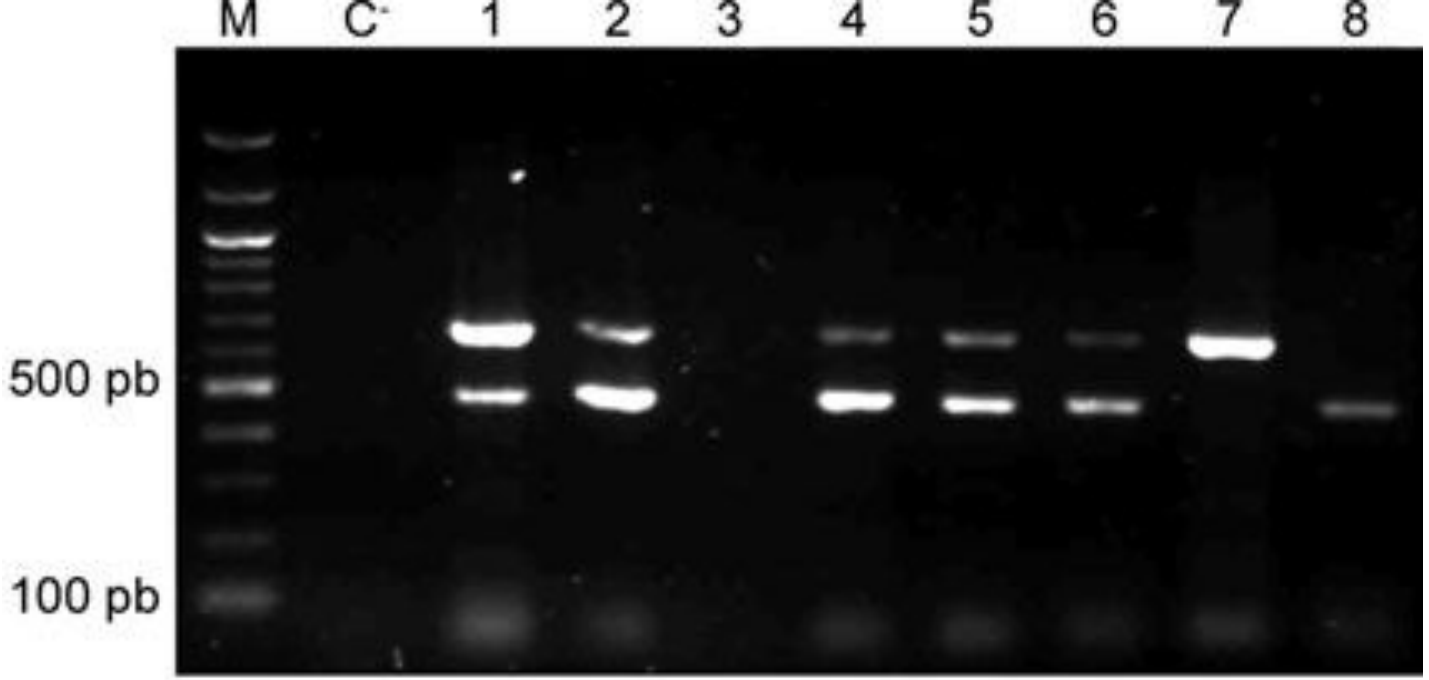
- There can be a little amount of nucleic acid and the DNA polymerase can amplify it even at high temperature,
  and be able to detect this nucleic acid.


1st cycle
2nd cycle
3rd cycle
30th cycle
Template DNA
(single copy)
1 Denaturation
2 Annealing
3 Extension
$2^{30} = 10^9$ copies
$2^1 = 2$ copies
$2^2 = 4$ copies
$2^3 = 8$ copies

Virus Isolation

– Nucleic acid methods
  - PCR (DNA), RT-PCR (RNA)
  - Can be used to detect viruses that are noncultivatable
  - Rapid identification (e.g. RT-PCR—4 Corners outbreak of hantavirus or FRET in the field)
  - Can be used to manage patients (e.g. HIV viral load)

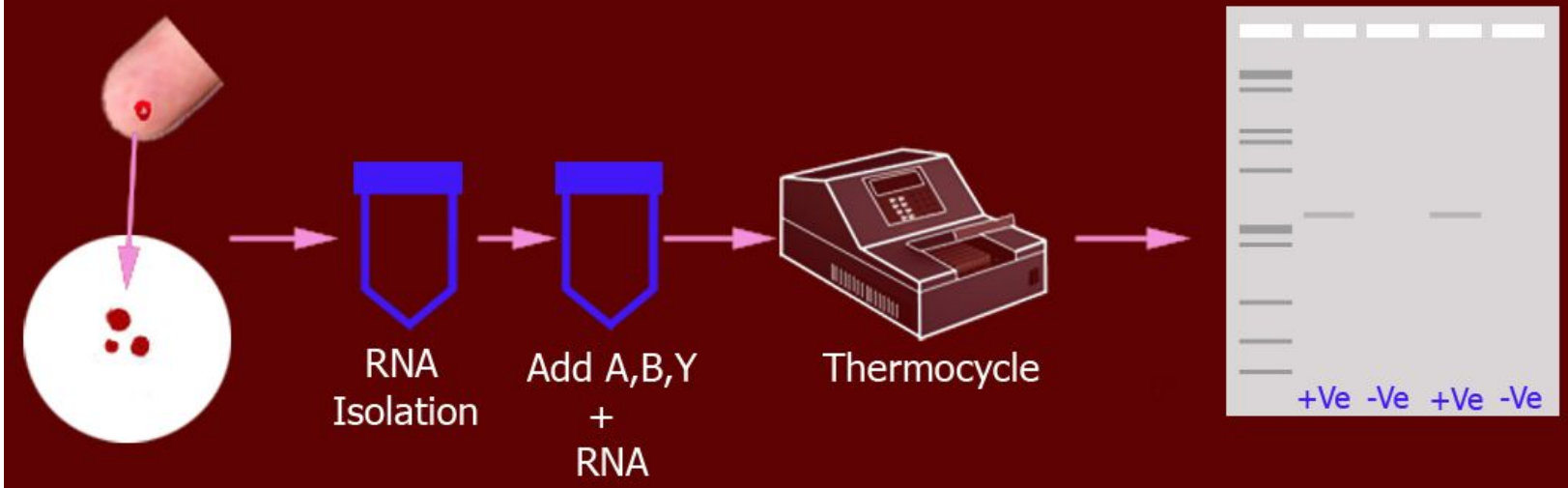




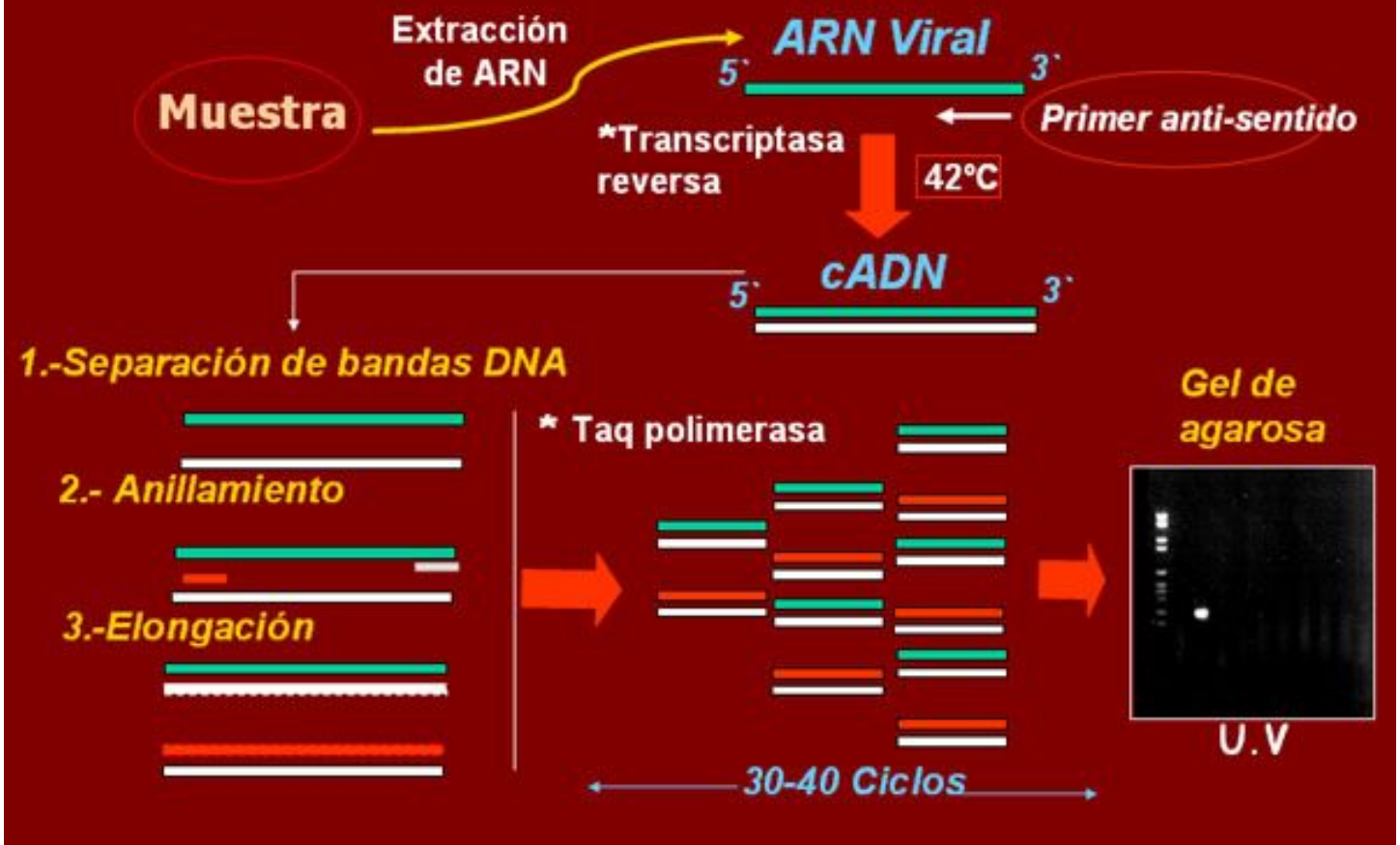
Duplex-PCR for the detection of human adenovirus (HAdV) and human respiratory syncytial virus (HRSV). Lanes M: molecular weight marker 100 bp ladder (GibcoBRL); C^+: positive control; C^-: negative control; 1, 2, 4-6: mixed samples (HRSV and HAdV); 3: negative sample; 7: HRSV prototype (conc. 200 $TCID_{50\%}$); 8: HAdV prototype (conc. 200 $TCID_{50\%}$).

Genekam Ebola PCR Kit

Box → Tube A,B,Y,D2,D1

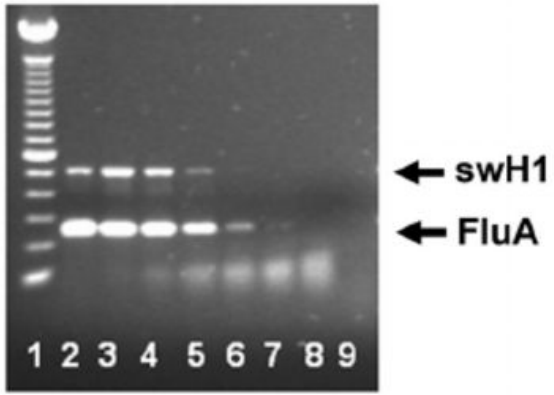


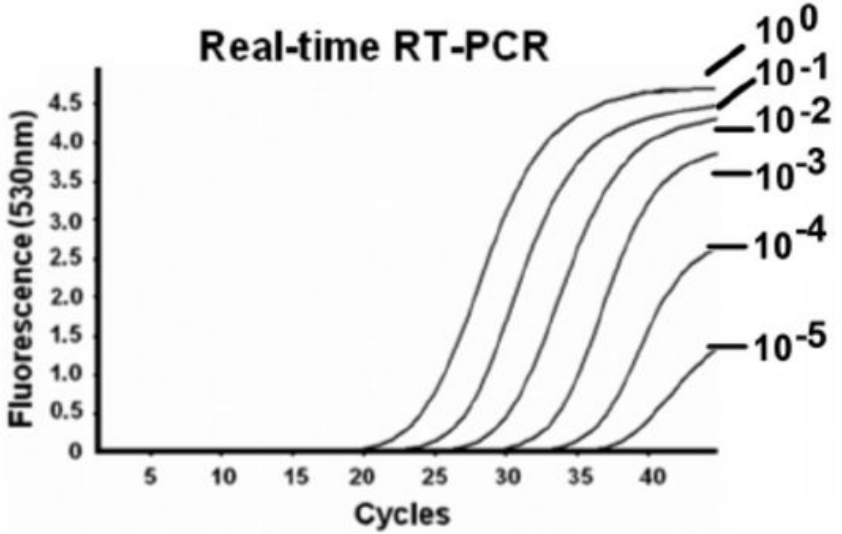
Detección de un VIRUS ARN por RT-PCR



A)

Influenza A + swH1 duplex

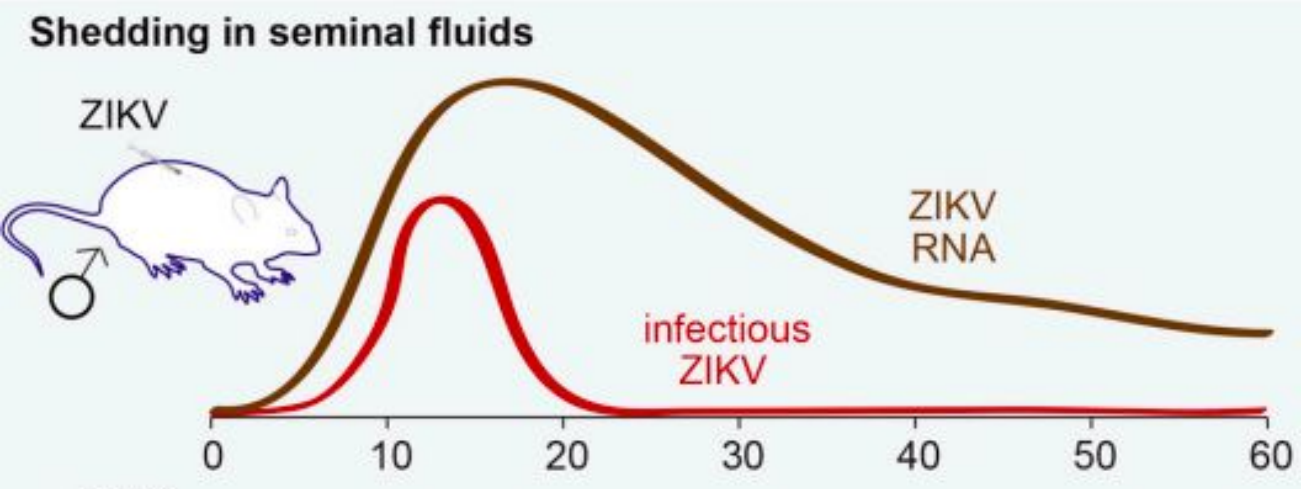




PCR product is not the same as infectious virus

- All we are looking for is a short piece of DNA made by the polymerase on a template.
- We never amplify the viral genome, it is very inefficient to do that.
- If you go to a physician and do a PCR test for influenza and the result is positive, you probably are infected with influenza because you have the right symptoms and it is the season.
- But a PCR positive result in research does not mean that there are infectious virus present.

PCR product is not the same as infectious virus



- But if you look at PCR of the seminal fluid, ZIKV is an RNA virus, so after reverse transcription, PCR is done.
- 60 days post the infection and ZIKV RNA is still detected
- PCR positive test does not mean the infectious virus is present

- They infected male mice subcutaneously injected with ZIKV
- Different days after infection, they took seminal fluids and look for infectious ZIKV by plaque assay.
- The peak of the infection is about 15 days post infection, then it is gone, no more infectious virus.

Deep, high-throughput sequencing

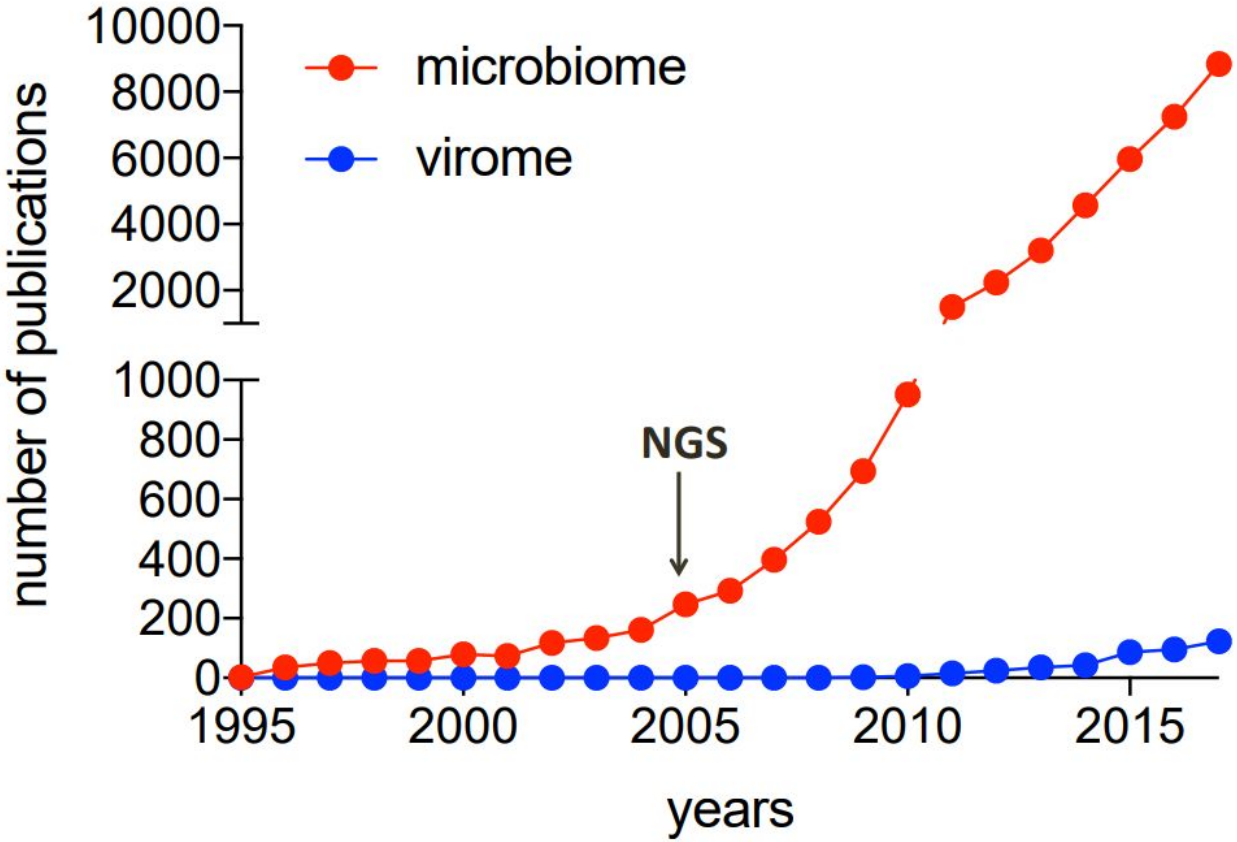
- The new sequencing methods (NGS) allow get 100 X coverage on a single molecule.
- Can detect many different sequences.
- Metagenomics is used to -identify new viruses in the environment
  -identify new pathogens.
- This made the human genome sequencing cheap, the first human genome sequence took 10 years and cost 3 billion $.
- Now you can have your DNA sequenced tomorrow by 1000 $; because the technology increased so much.

Enteric Virome Analysis of Non-Invasive Samples From Gorillas by Next-Generation Sequencing and Association With SIV Infection

Mirela D'arc[1,2], Carolina Furtado[1], Juliana D. Siqueira[1], Ahidjo Ayouba[3], Martine Peeters[3], Marcelo A. Soares[1,2]

1 Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil
2 Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil
3 Institut de Recherche pour le Développement (IRD), Montpellier, France

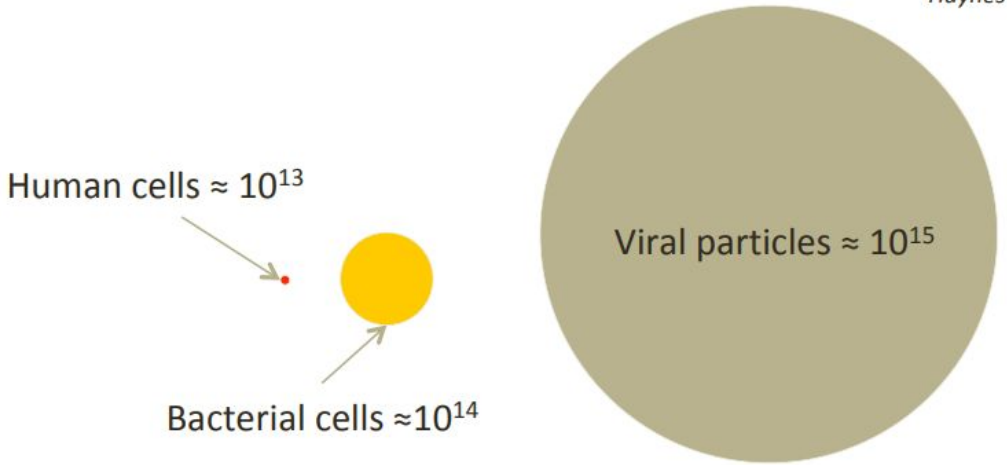
The virome, a neglected part of the microbiome...



Viruses and humans

- ✓ It is estimated that there are 100 times more viruses in our body than human cells

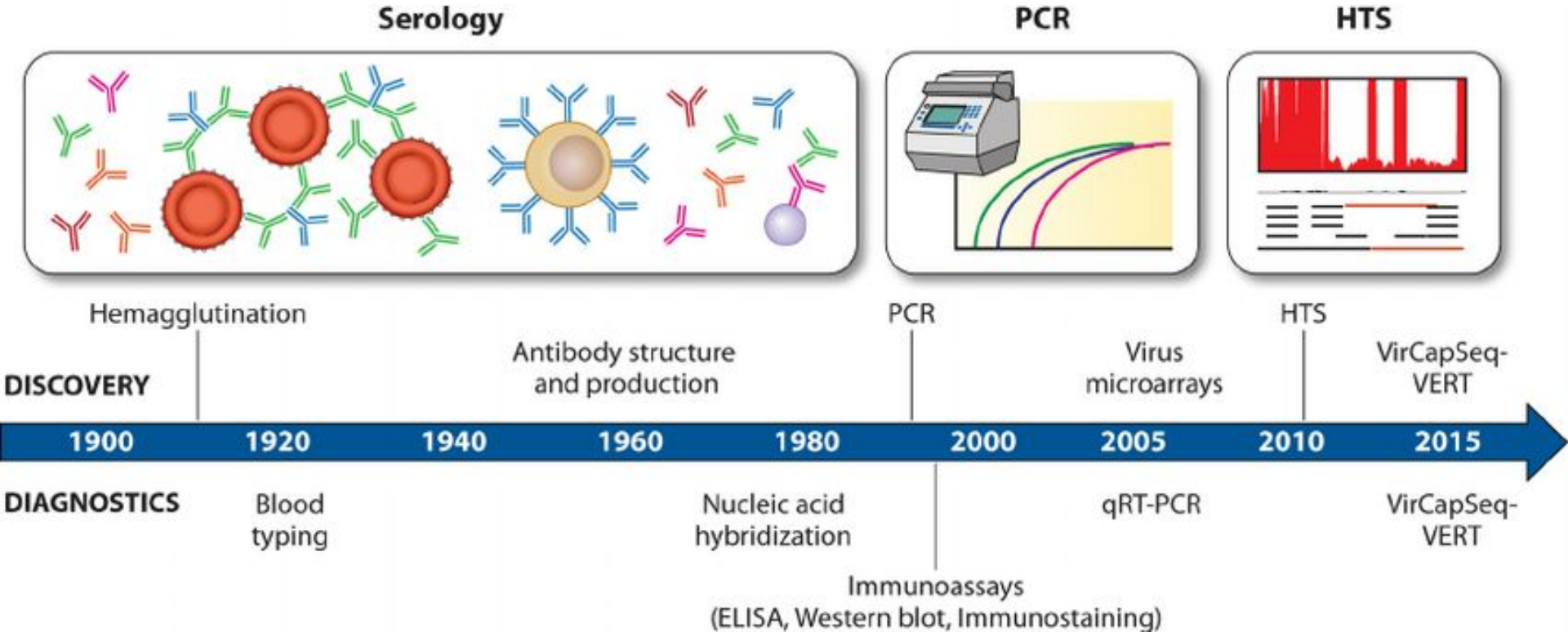
Haynes & Rohwer (2010)



- ✓ The number of free virions varies from 10^9 particles/g for body barriers (gut, oropharynx, skin) to 10^7 and 10^5 particles/ml for urine and blood, respectively

Haynes & Rohwer (2010)
Mokili, Curr. Op. Virol. (2012)

- ✓ Collectively, this viral flora is known as the **human virome**


Serology
PCR
HTS
Hemagglutination
PCR
HTS
DISCOVERY
Antibody structure and production
Virus microarrays
VirCapSeq-VERT
1900
1920
1940
1960
1980
2000
2005
2010
2015
DIAGNOSTICS
Blood typing
Nucleic acid hybridization
qRT-PCR
VirCapSeq-VERT
Immunoassays
(ELISA, Western blot, Immunostaining)