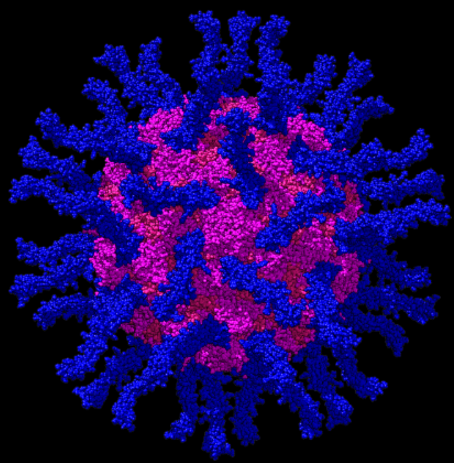


VIROLOGY LIVE

WITH VINCENT RACANIELLO

The Infectious Cycle

Session 2

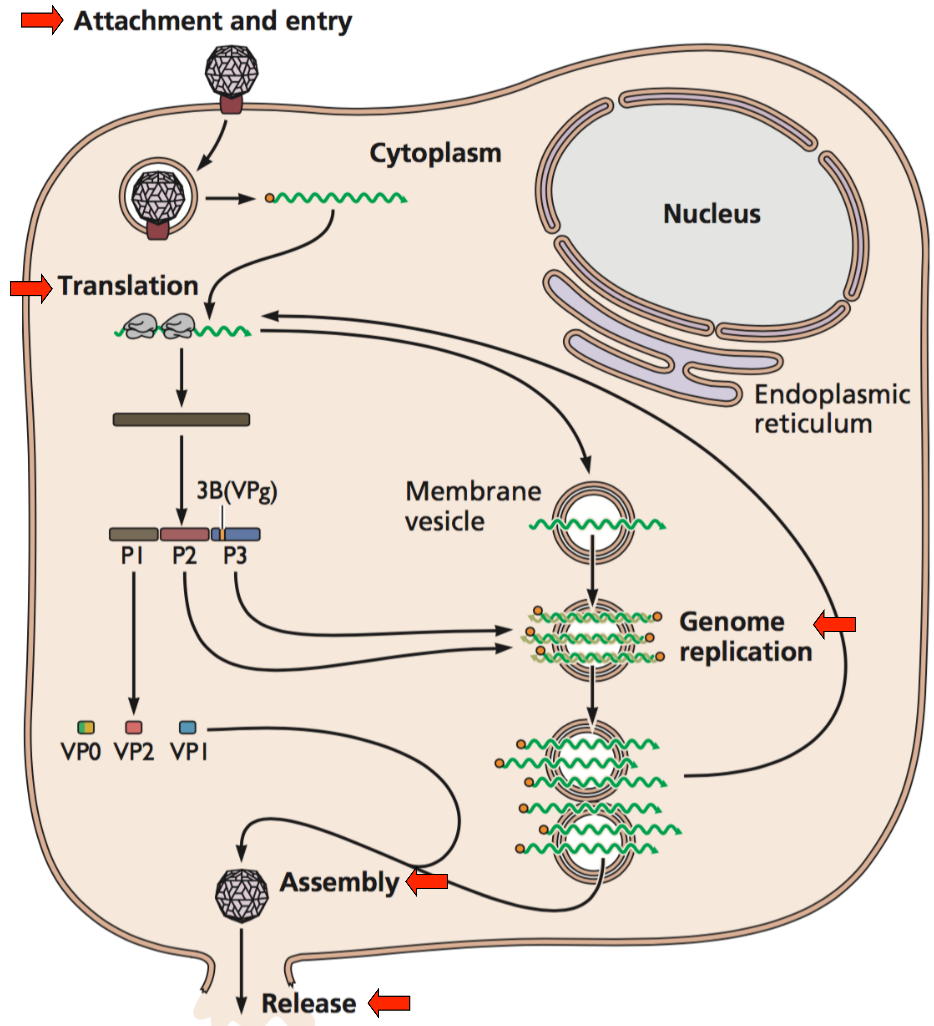
Virology Live

Fall 2021

"You know my methods, Watson"
--Sir Arthur Conan Doyle

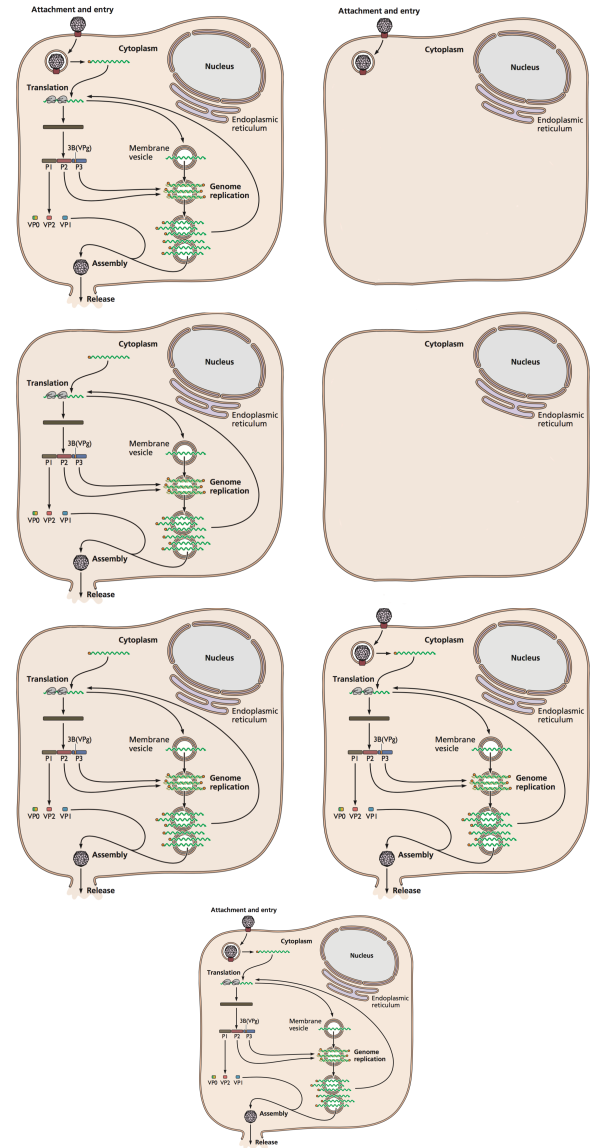
The Infectious Cycle

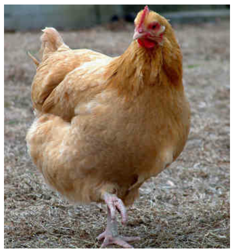
Virologists divide the infectious cycle into steps to facilitate their study, but no such artificial boundaries occur



Some important definitions

- A **susceptible** cell has a functional receptor for a given virus - *the cell may or may not be able to support viral replication*
- A **resistant** cell has no receptor - *it may or may not be competent to support viral replication*
- A **permissive** cell has the capacity to replicate virus - *it may or may not be susceptible*
- A **susceptible** AND **permissive** cell is the only cell that can take up a virus particle and replicate it

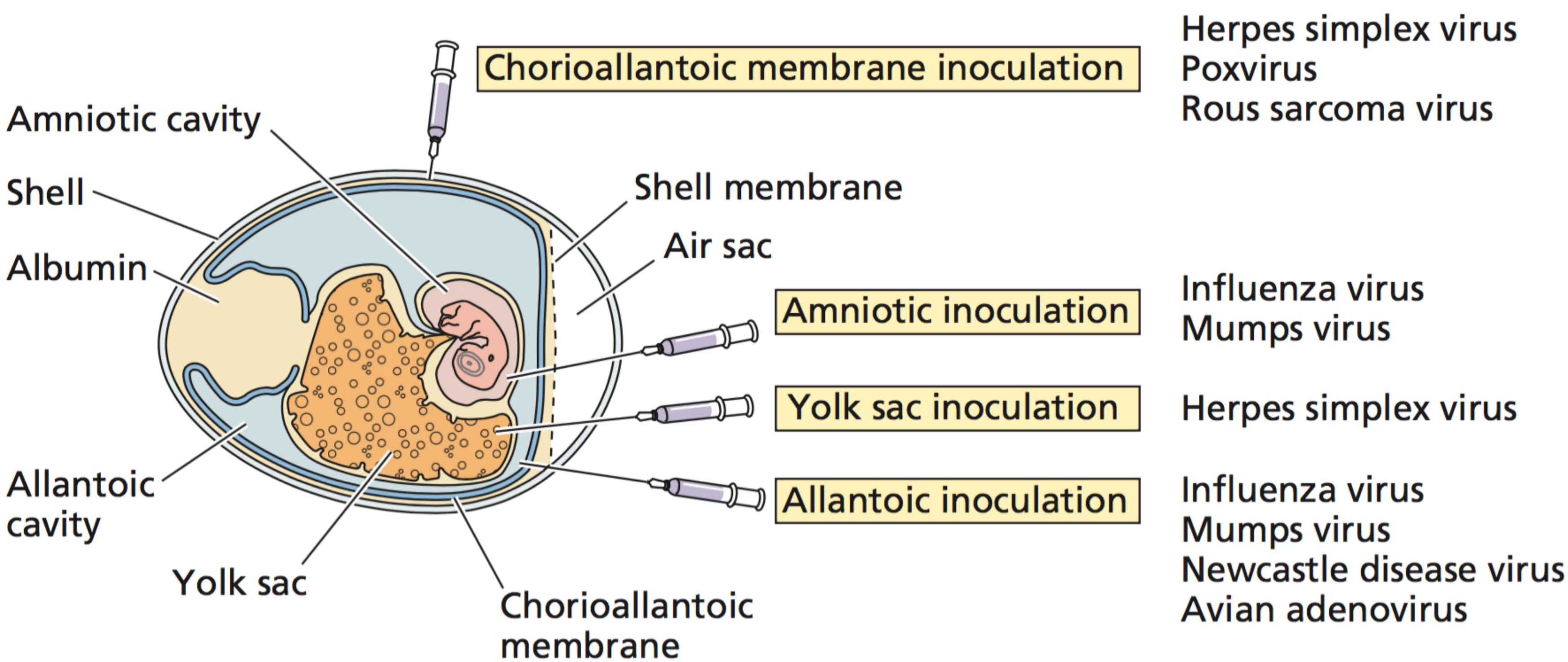




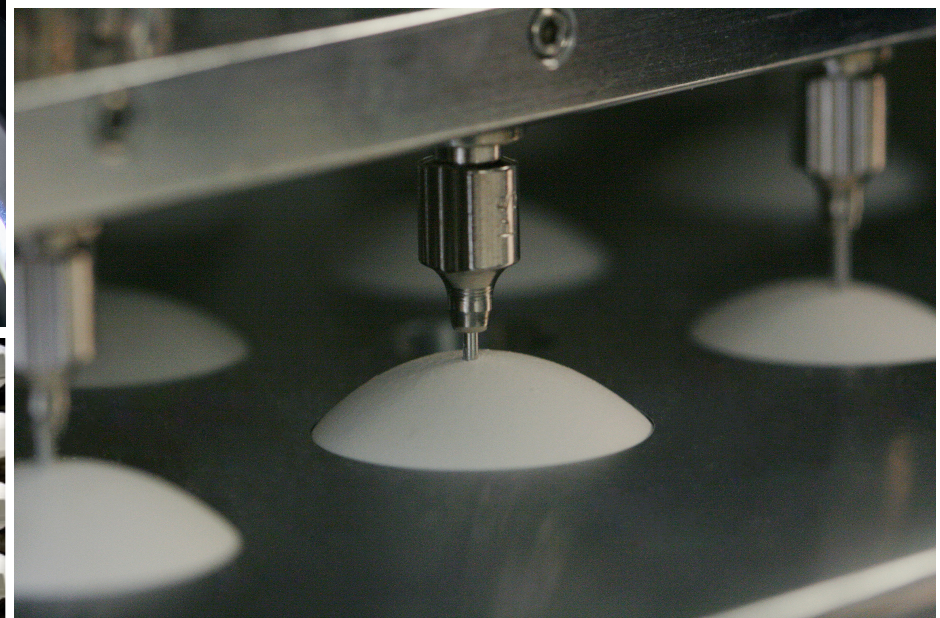


- Animal viruses at first could not be routinely propagated in cultured cells
- Most viruses were grown in laboratory animals





Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Albumin
Shell membrane
Air sac
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle disease virus
Avian adenovirus
Allantoic cavity
Yolk sac
Chorioallantoic membrane

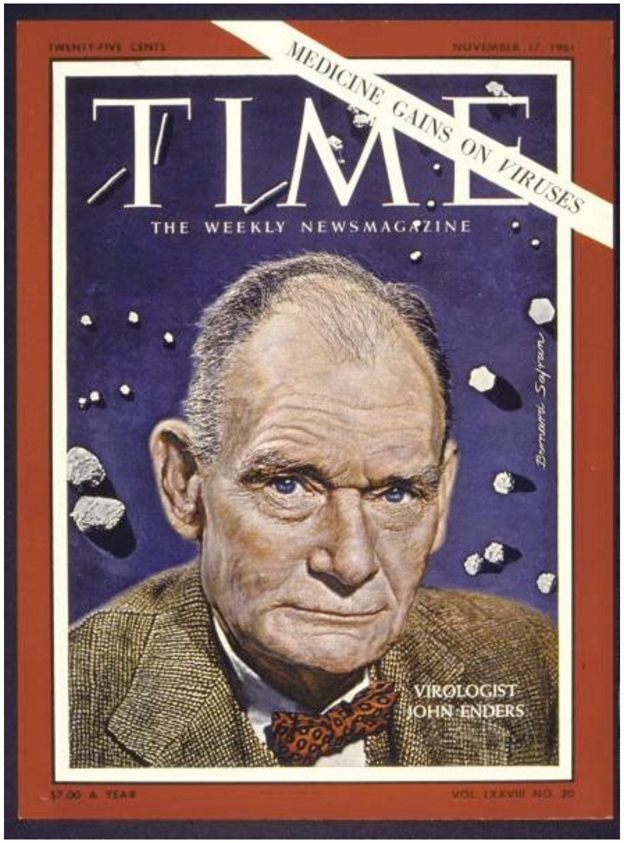
www.vaccinews.net



www.news.sanofi.us

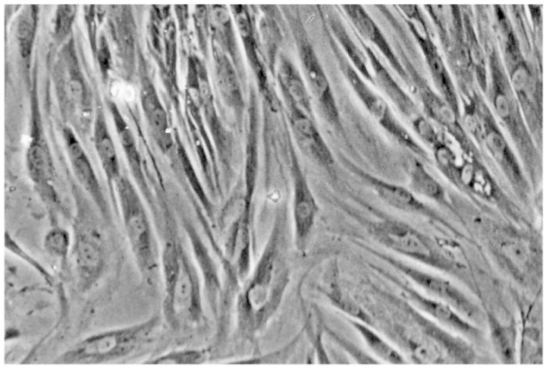
Studying the infectious cycle in cells

- Not possible before 1949 (animal viruses)
- *Enders, Weller, Robbins* propagate poliovirus in human cell culture - primary cultures of embryonic tissues
- Nobel prize, 1954



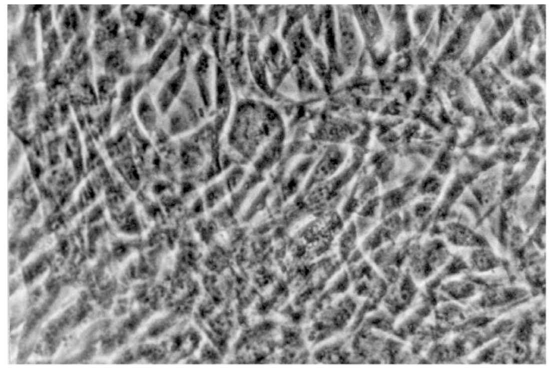
Virus cultivation

A



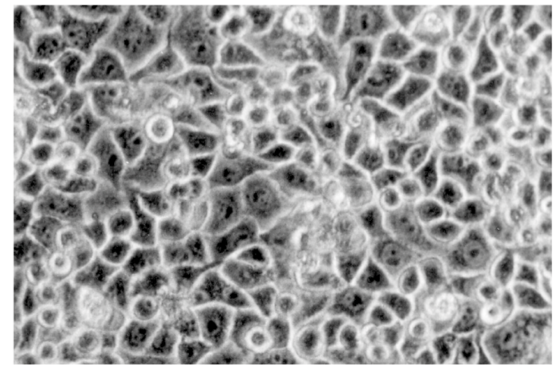
Primary human
foreskin fibroblasts

B



Mouse fibroblast
cell line (3T3)

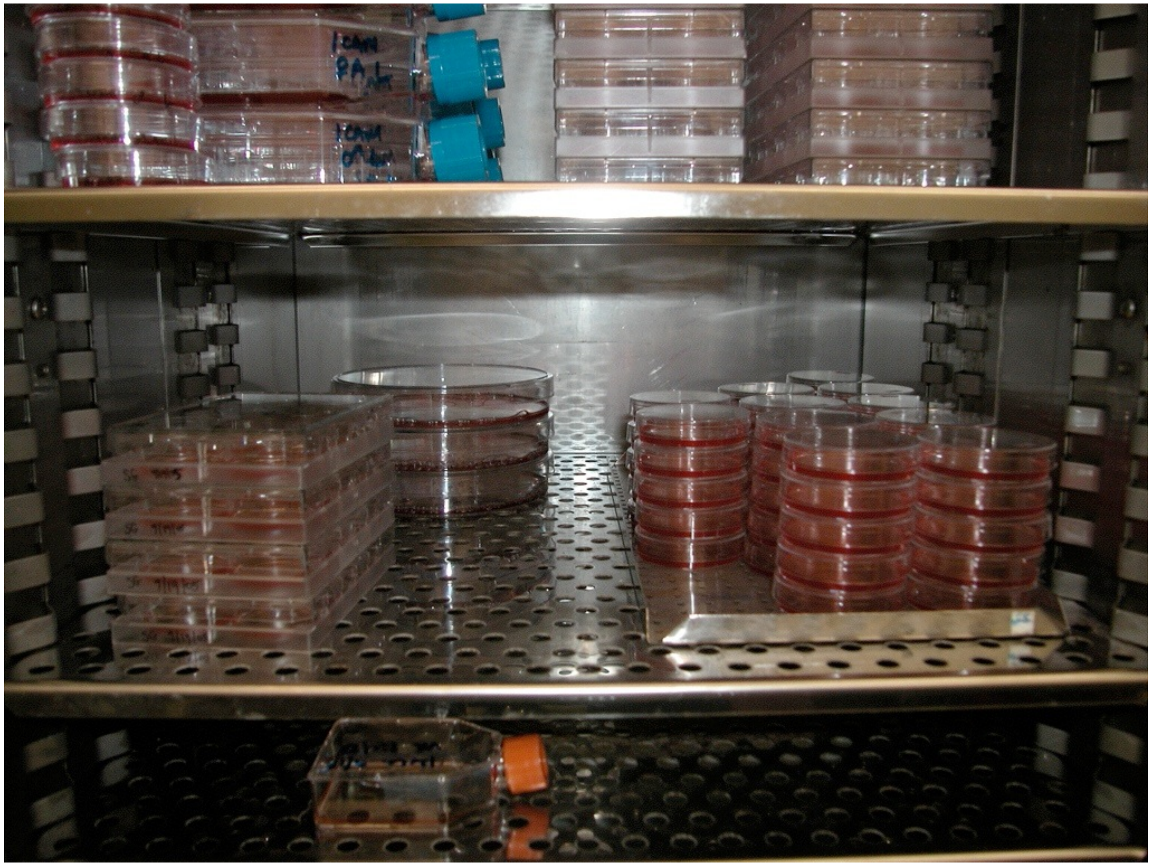
C



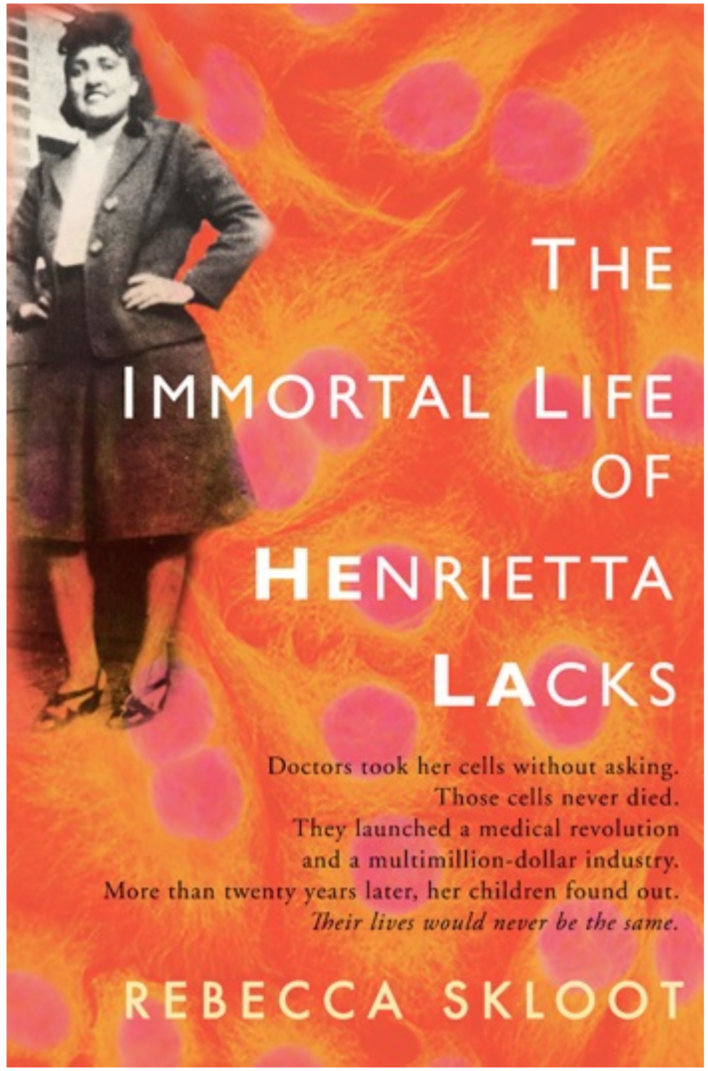
Human epithelial
cell line (HeLa)

continuous cell lines

Diploid cell strains (e.g. WI-38, human embryonic lung)



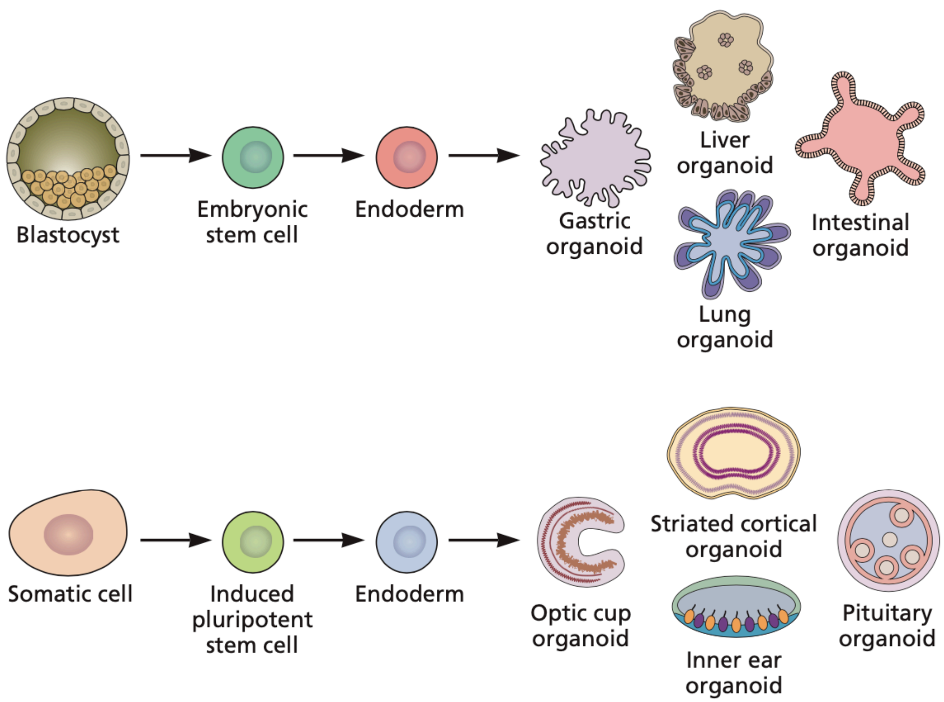

9/9
SPINNER FLASK
1965-01000



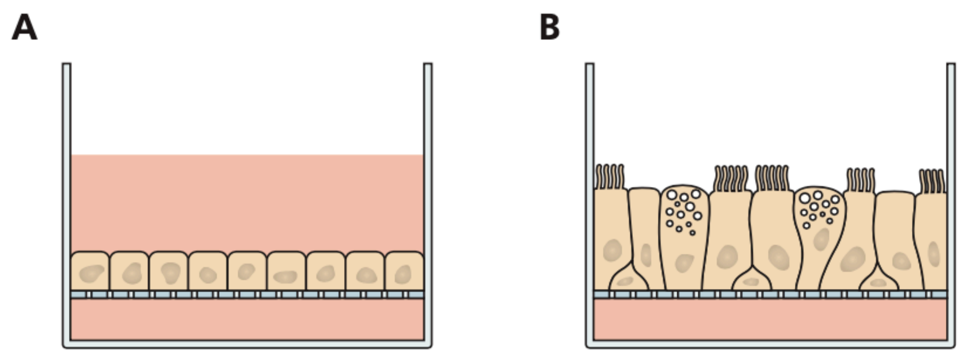
http://www.virology.ws/2009/02/09/the-amazing-hela-cells-of-henrietta-lacks/

Amazing advances in cell culture

Organoid cultures



Air-liquid interface cultures



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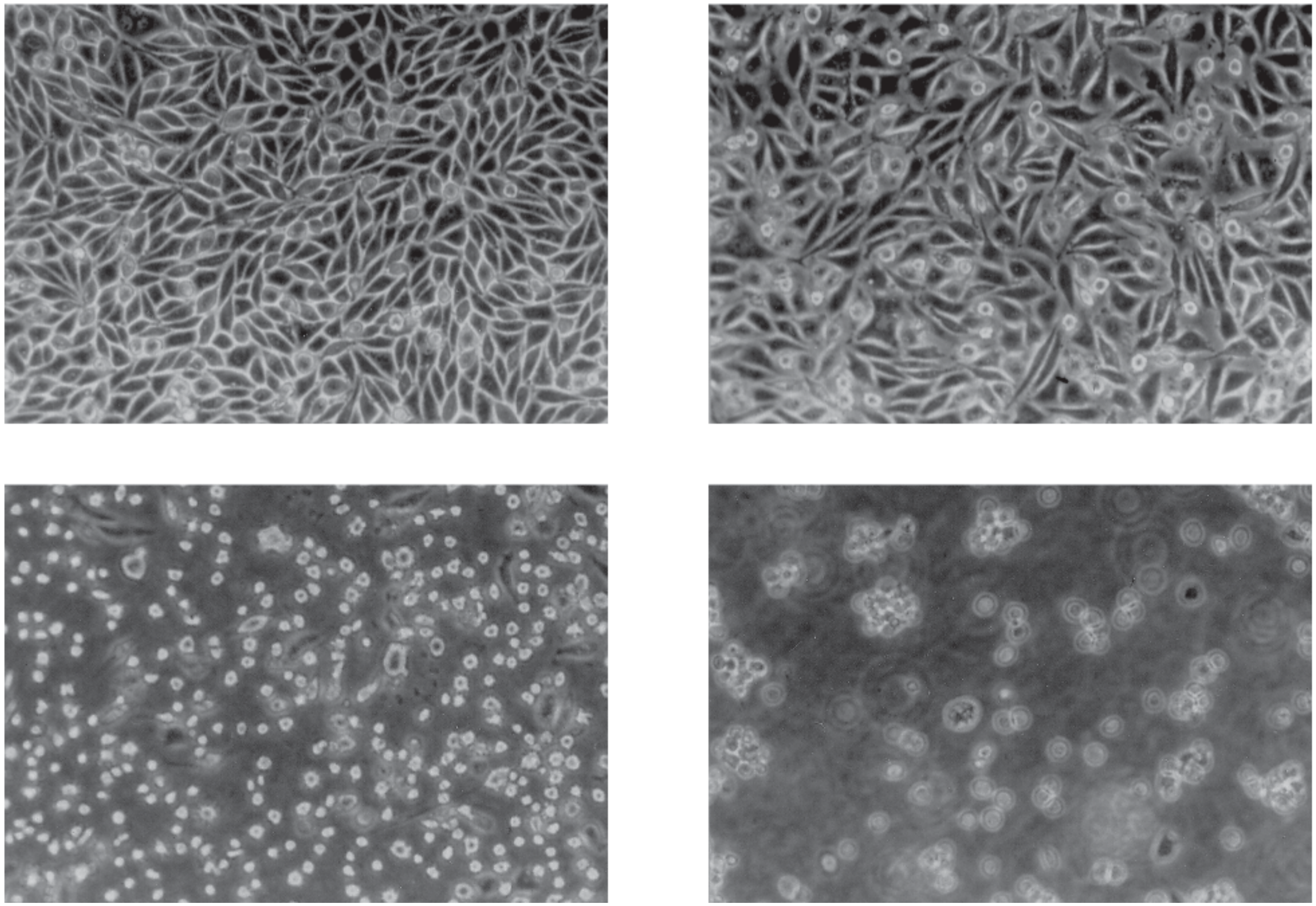
A _________ and _________ cell is the only cell that can take up a virus particle and replicate it (fill in the blanks)

A. Naive and resistant
B. Primary and permissive
C. Susceptible and permissive
D. Susceptible and naive
E. Continuous and immortal



Cytopathic effect (CPE)

Article

A pneumonia outbreak associated with a new coronavirus of probable bat origin

https://doi.org/10.1038/s41586-020-2012-7

Received: 20 January 2020

Accepted: 29 January 2020

Published online: 3 February 2020

Open access

Check for updates

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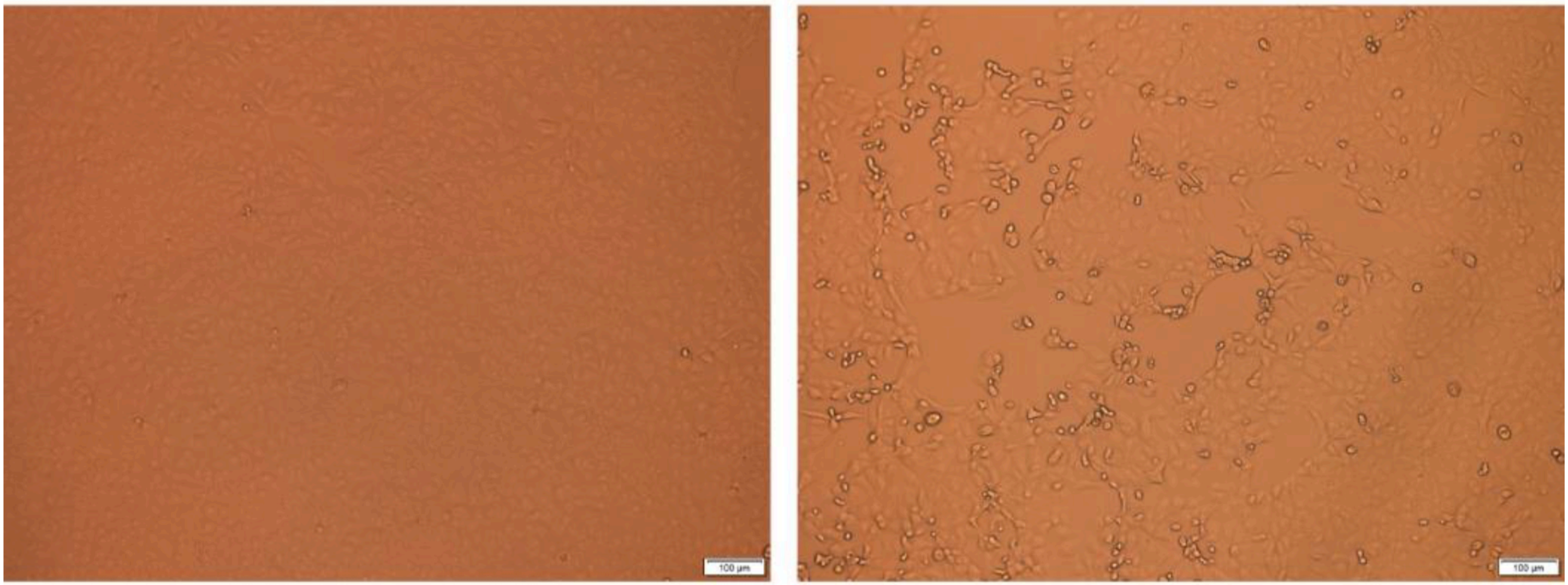
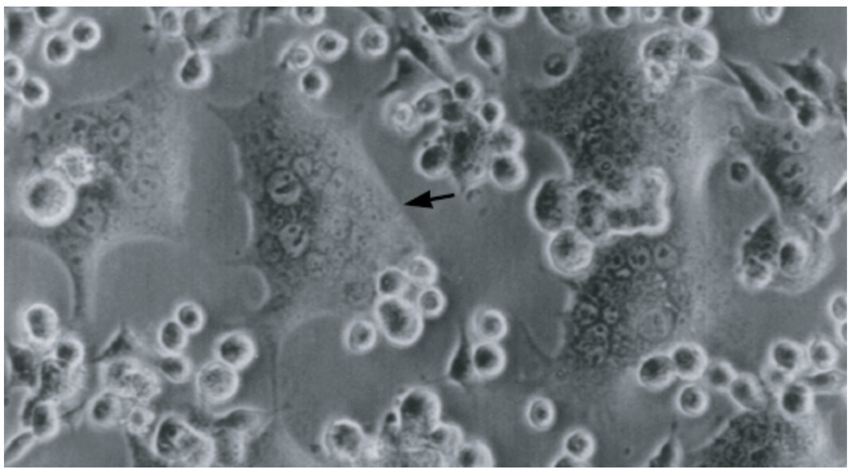
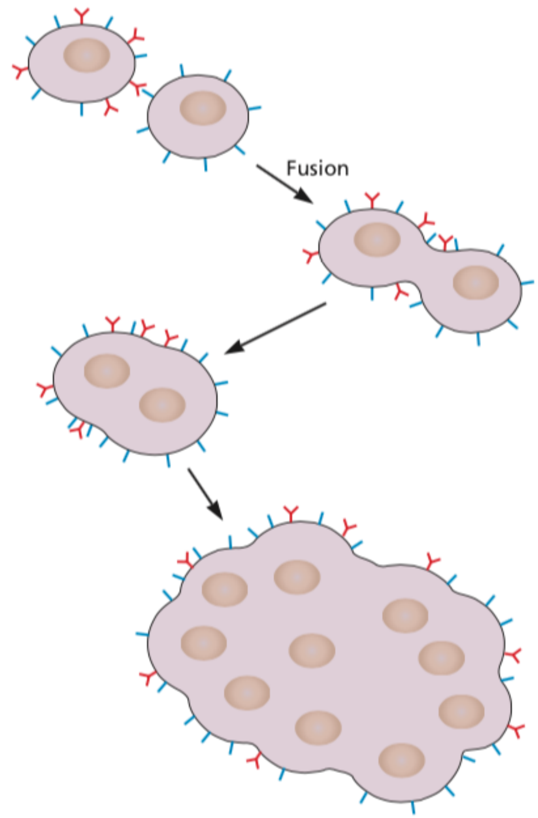
Since the outbreak of severe acute respiratory syndrome (SARS) 18 years ago, a large number of SARS-related coronaviruses (SARSr-CoVs) have been discovered in their natural reservoir host, bats[1–4]. Previous studies have shown that some bat SARSr-CoVs have the potential to infect humans[5–7]. Here we report the identification and characterization of a new coronavirus (2019-nCoV), which caused an epidemic of acute respiratory syndrome in humans in Wuhan, China. The epidemic, which started on 12 December 2019, had caused 2,794 laboratory-confirmed infections including 80 deaths by 26 January 2020. Full-length genome sequences were obtained from five patients at an early stage of the outbreak. The sequences are almost identical and share 79.6% sequence identity to SARS-CoV. Furthermore, we show that 2019-nCoV is 96% identical at the whole-genome level to a bat coronavirus. Pairwise protein sequence analysis of seven conserved non-structural proteins domains show that this virus belongs to the species of *SARSr-CoV*. In addition, 2019-nCoV virus isolated from the bronchoalveolar lavage fluid of a critically ill patient could be neutralized by sera from several patients. Notably, we confirmed that 2019-nCoV uses the same cell entry receptor—angiotensin converting enzyme II (ACE2)—as SARS-CoV.

We then successfully isolated the virus (named nCoV-2019 BetaCoV/Wuhan/WIV04/2019), in Vero and Huh7 cells using BALF sample from ICU-06 patient. Clear cytopathogenic effects were observed in cells after three days incubation (Extended Data Figure 5a and 5b). The identity of the strain WIV04 was



Formation of syncytia





Examples of cytopathic effects

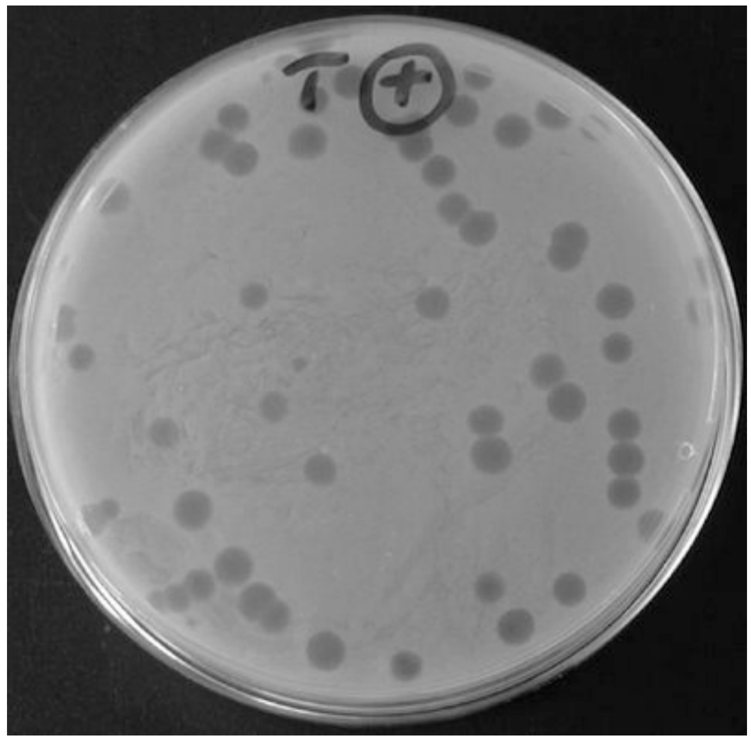
Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Polyomaviruses, papillomaviruses
Syncytium formation (cell fusion)	Paramyxoviruses, coronaviruses
Margination and breaking of chromosomes	Herpesviruses
Rounding up and detachment of cultured cells	Herpesviruses, rhabdoviruses, adenoviruses, picornaviruses
Inclusion bodies	
Virions in nucleus	Adenoviruses
Virions in cytoplasm (Negri bodies)	Rabies virus
"Factories" in cytoplasm (Guarnieri bodies)	Poxviruses
Clumps of ribosomes in virions	Arenaviruses
Clumps of chromatin in nucleus	Herpesviruses

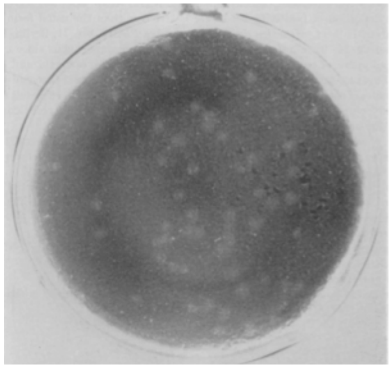
How many viruses in a sample?

- Infectivity
- Physical: virus particles and their components

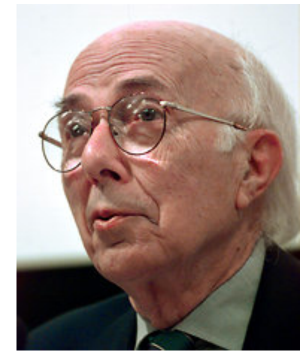
Plaque assay

1930s: used to study multiplication of bacteriophages





Plaque assay



1952, Renato Dulbecco developed plaque assay for animal viruses

Nobel Prize, 1975

PRODUCTION OF PLAQUES IN MONOLAYER TISSUE CULTURES BY SINGLE PARTICLES OF AN ANIMAL VIRUS

By Renato Dulbecco

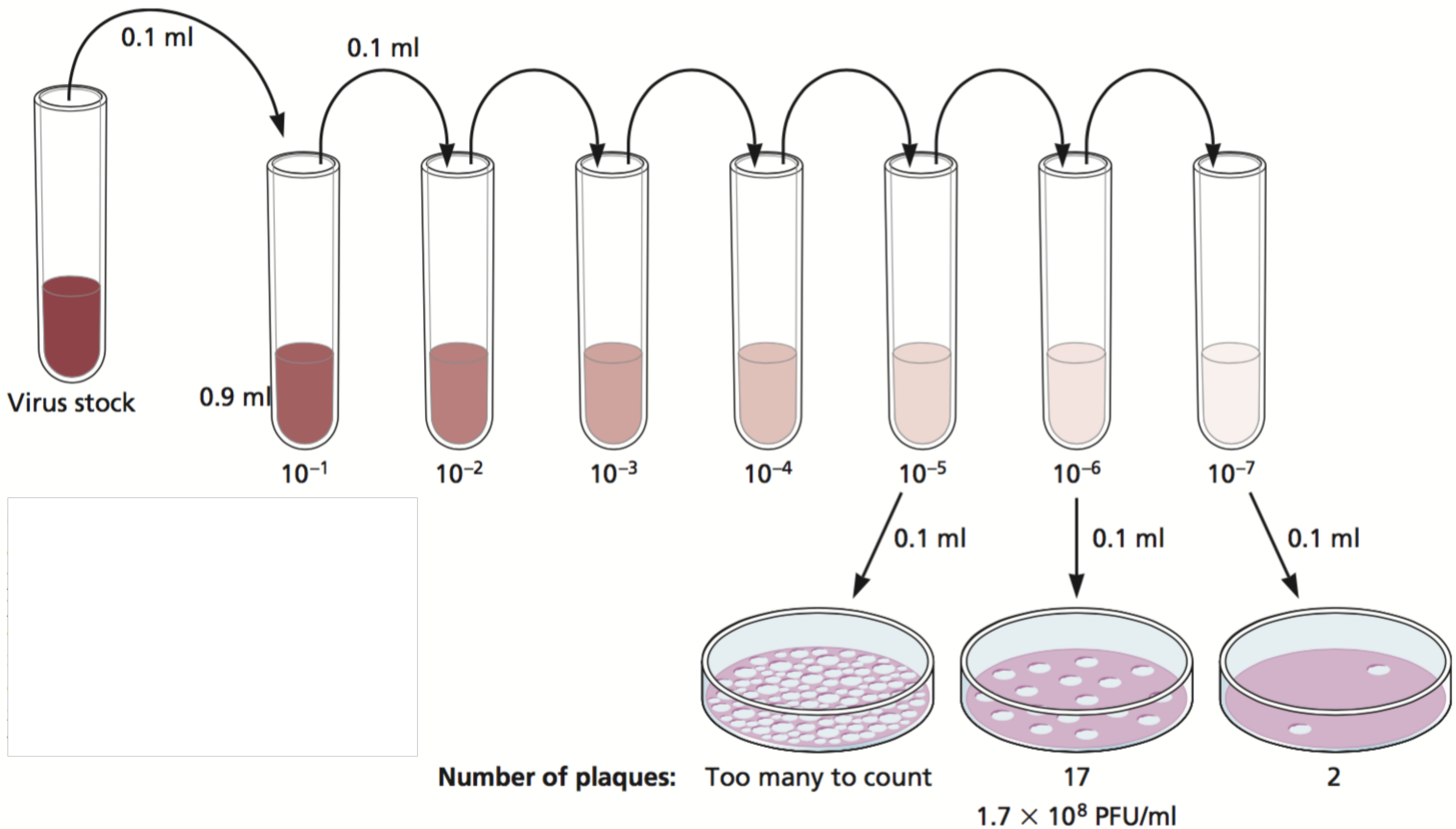
California Institute of Technology, Pasadena, California

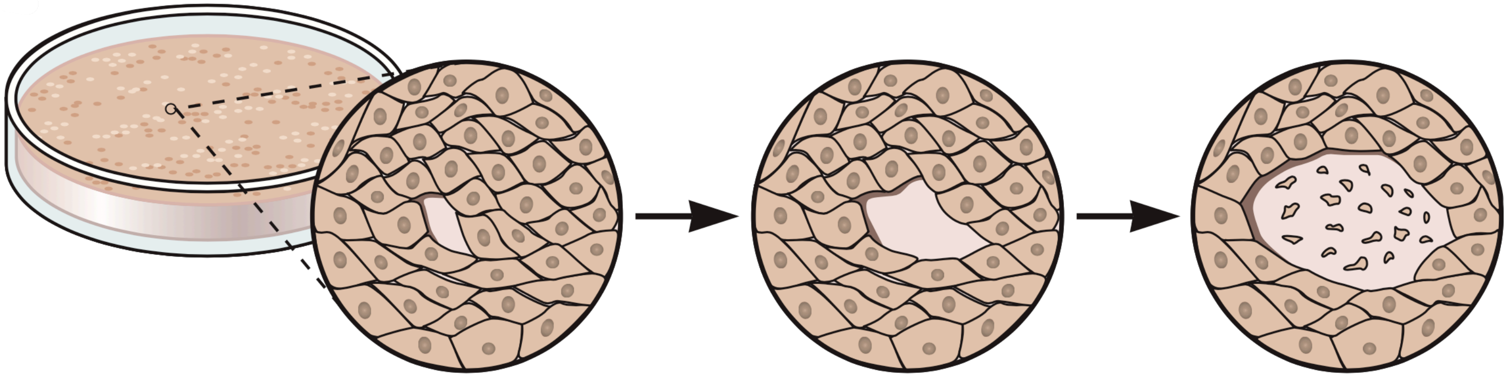
Read before the Academy, April 29, 1952

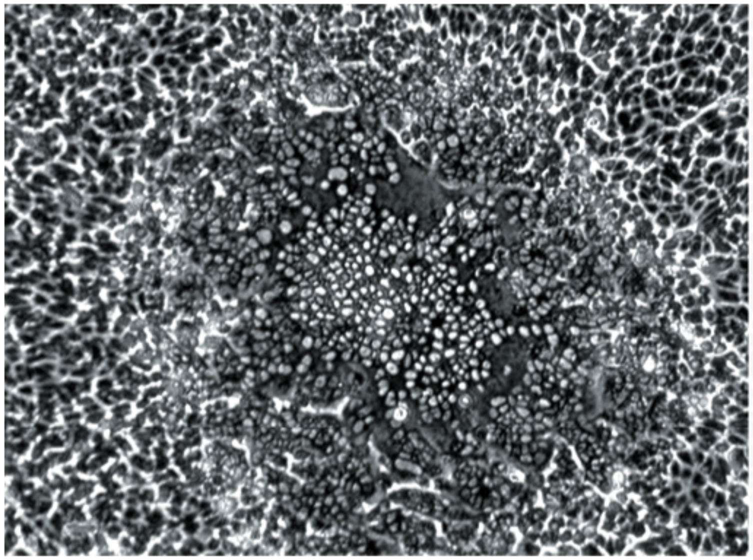
Research on the growth characteristics and genetic properties of animal viruses has stood greatly in need of improved quantitative techniques, such as those used in the related field of bacteriophage studies.

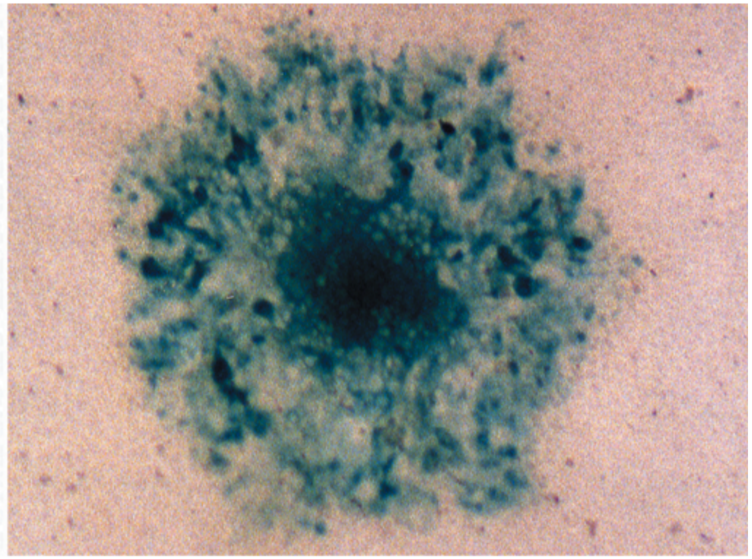
The requirements for a quantitative virus technique are as follows: (1) The use of a uniform type of host cell; (2) an accurate assay technique; (3) the isolation of the progeny of a single virus particle; and (4) the separate isolation of each of the virus particles produced by a single infected

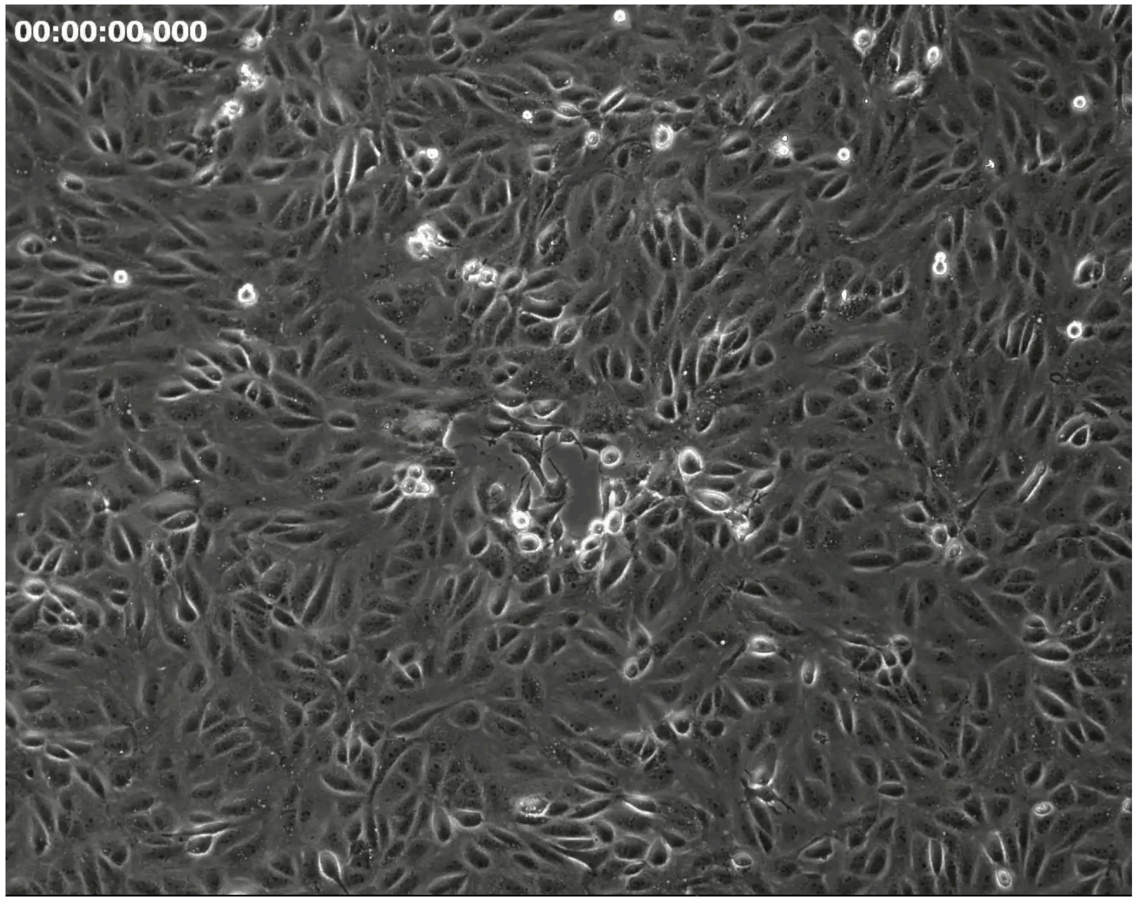
Plaque assay










00:00:00.000


THE WALL OF POLIO
MICROBES WE NEED THEM
SMALL TOWN
BIG
COLUMBI
EST. 1754
UNIVERSITY
NEW YORK CITY

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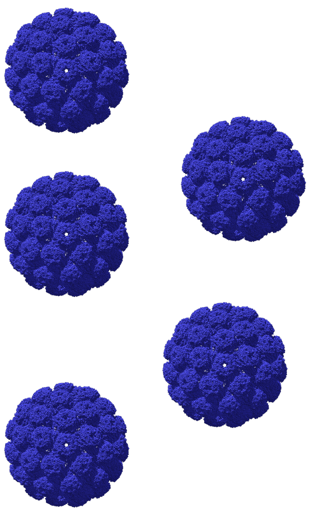
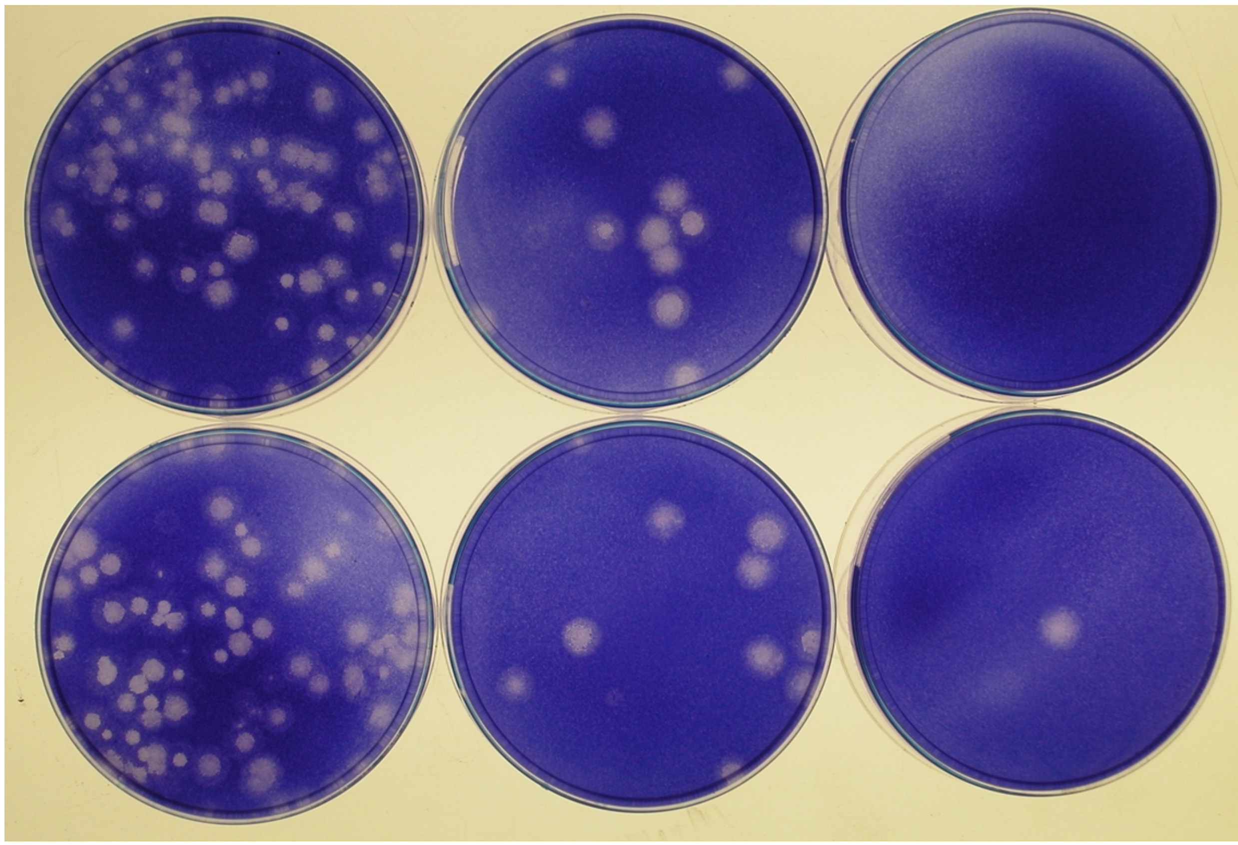
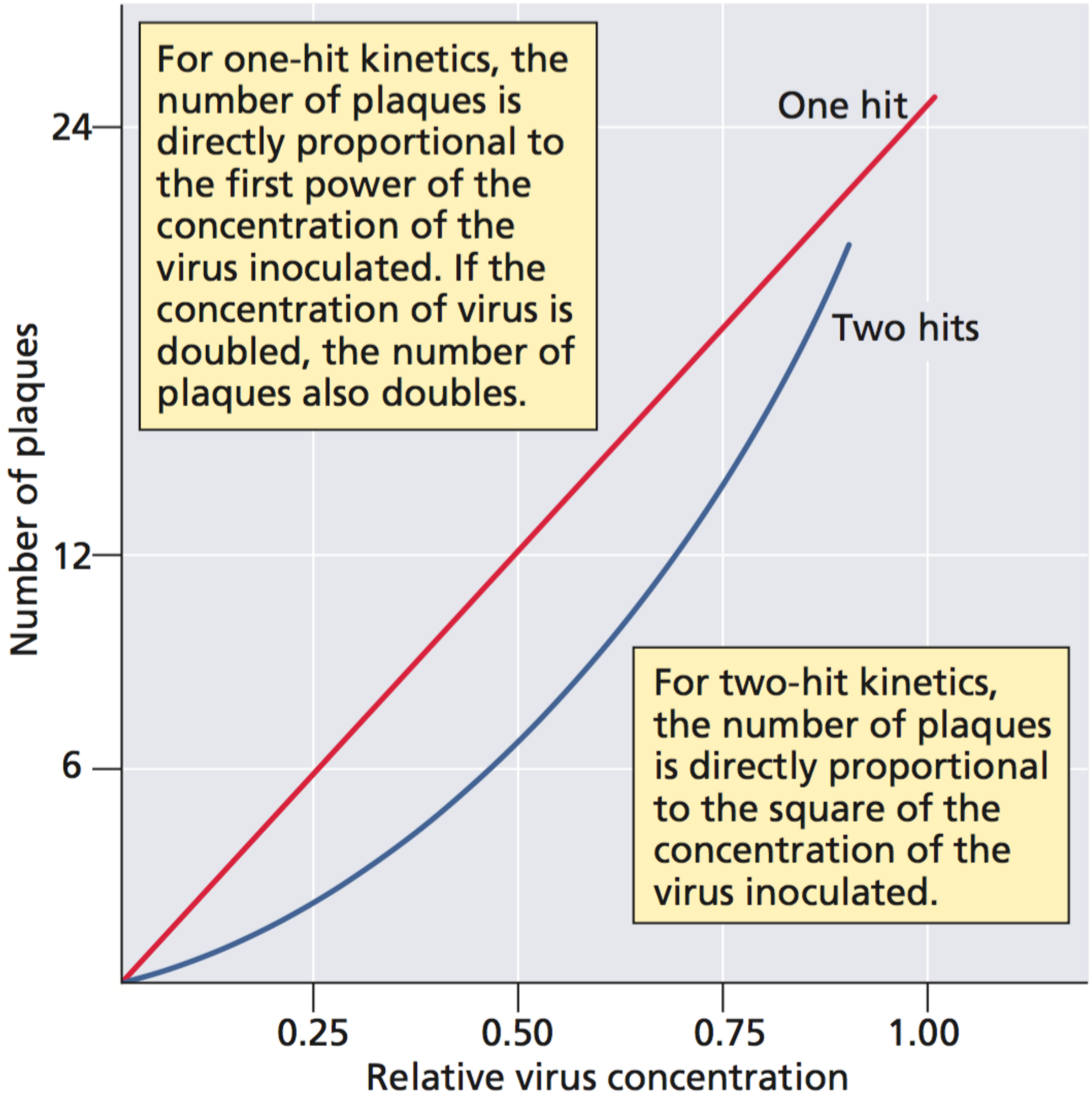
When doing a plaque assay, what is the purpose of adding a semi-solid agar overlay on the monolayer of infected cells?

A. To stabilize progeny virus particles
B. To ensure that cells remain susceptible and permissive
C. To act as a pH indicator
D. To keep cells adherent to the plate during incubation
E. To restrict viral diffusion after lysis of infected cells

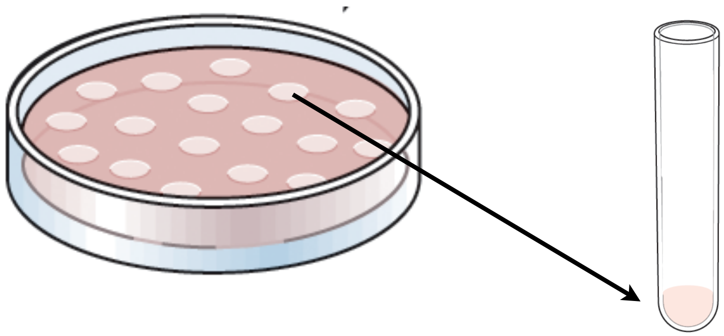
How many viruses are needed to form a plaque?






For one-hit kinetics, the number of plaques is directly proportional to the first power of the concentration of the virus inoculated. If the concentration of virus is doubled, the number of plaques also doubles.
One hit
Two hits
For two-hit kinetics, the number of plaques is directly proportional to the square of the concentration of the virus inoculated.
Number of plaques
24
12
6
0.25
0.50
0.75
1.00
Relative virus concentration

Plaque purification

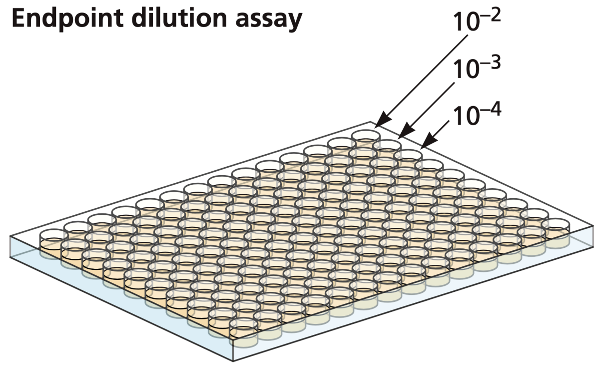


A method for producing virus stocks

Usually done 3 times

For viruses that do not form plaques: Endpoint dilution assay

Endpoint dilution assay



TCID$_{50}$

Virus dilution	Cytopathic effect									
10^{-2}	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	+	+	+	+	+	+	+	+	+
10^{-4}	+	+	−	+	+	+	+	+	+	+
10^{-5}	−	+	+	−	+	−	−	+	−	+
10^{-6}	−	−	−	−	−	−	+	−	−	−
10^{-7}	−	−	−	−	−	−	−	−	−	−

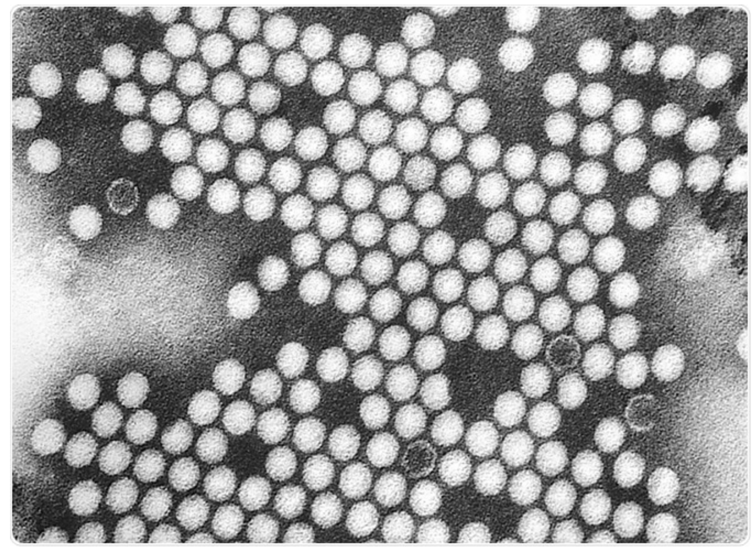
Not all virus particles are infectious!

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

$$\frac{\text{\# of } \textit{physical} \text{ particles}}{\text{\# of } \textit{infectious} \text{ particles}}$$

Particle-to-PFU ratio

- # of *physical* particles ÷ # of *infectious* particles
- A single particle *can* initiate infection
- Not all viruses are successful
  - Damaged particles
  - Mutations
  - Complexity of infectious cycle
- Complicates study



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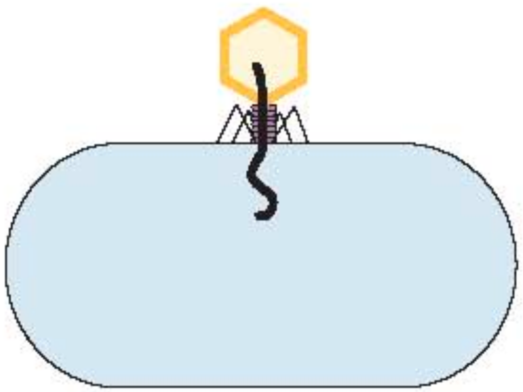
In the ‘particle to pfu ratio’, ‘particle’ can best be described as:

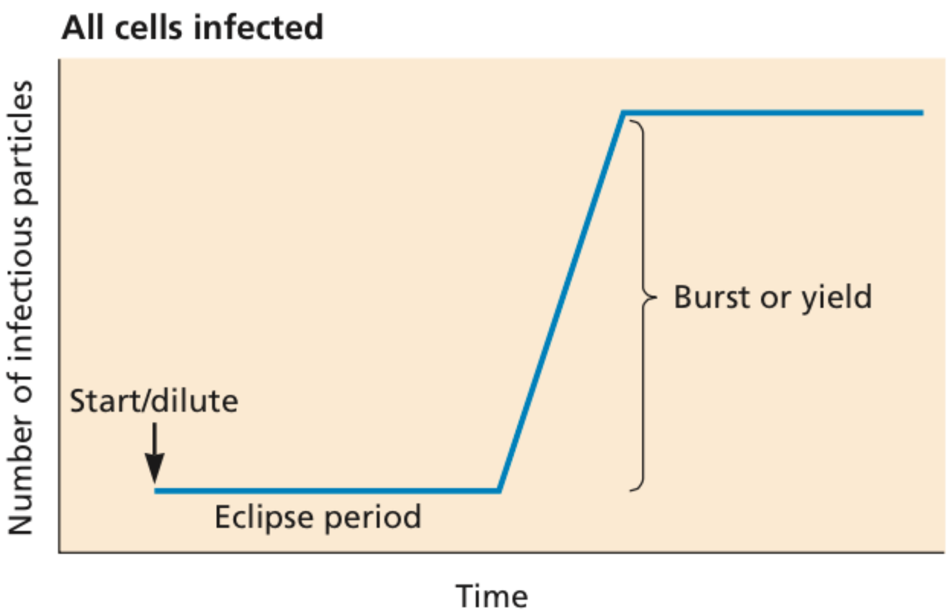
A. One of the proteins which makes up the virus
B. A virus which may or may not be infectious
C. A virus which is infectious
D. A virus which is not infectious
E. Elementary or composite

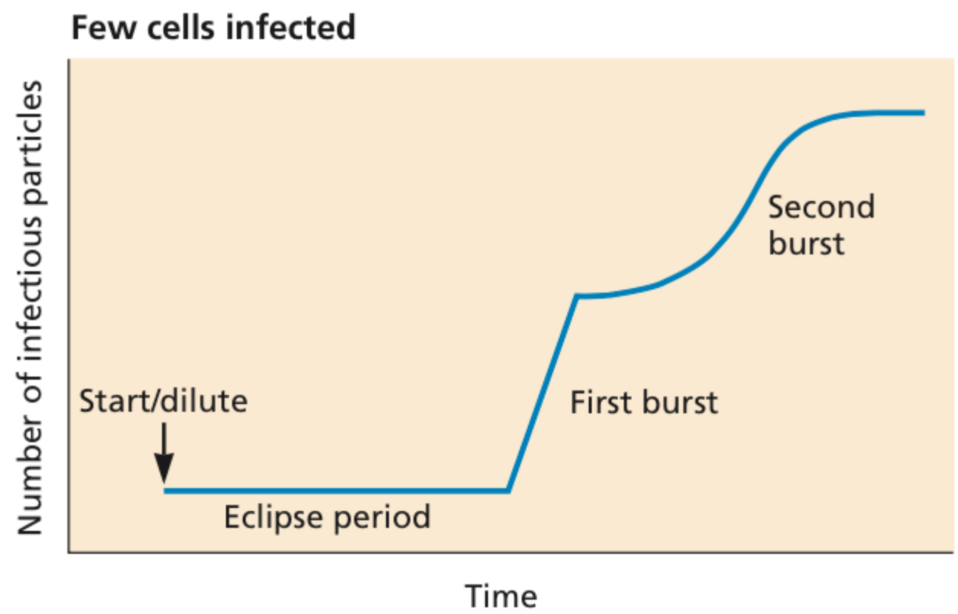
One-step growth cycle:
A method to study virus reproduction in cells

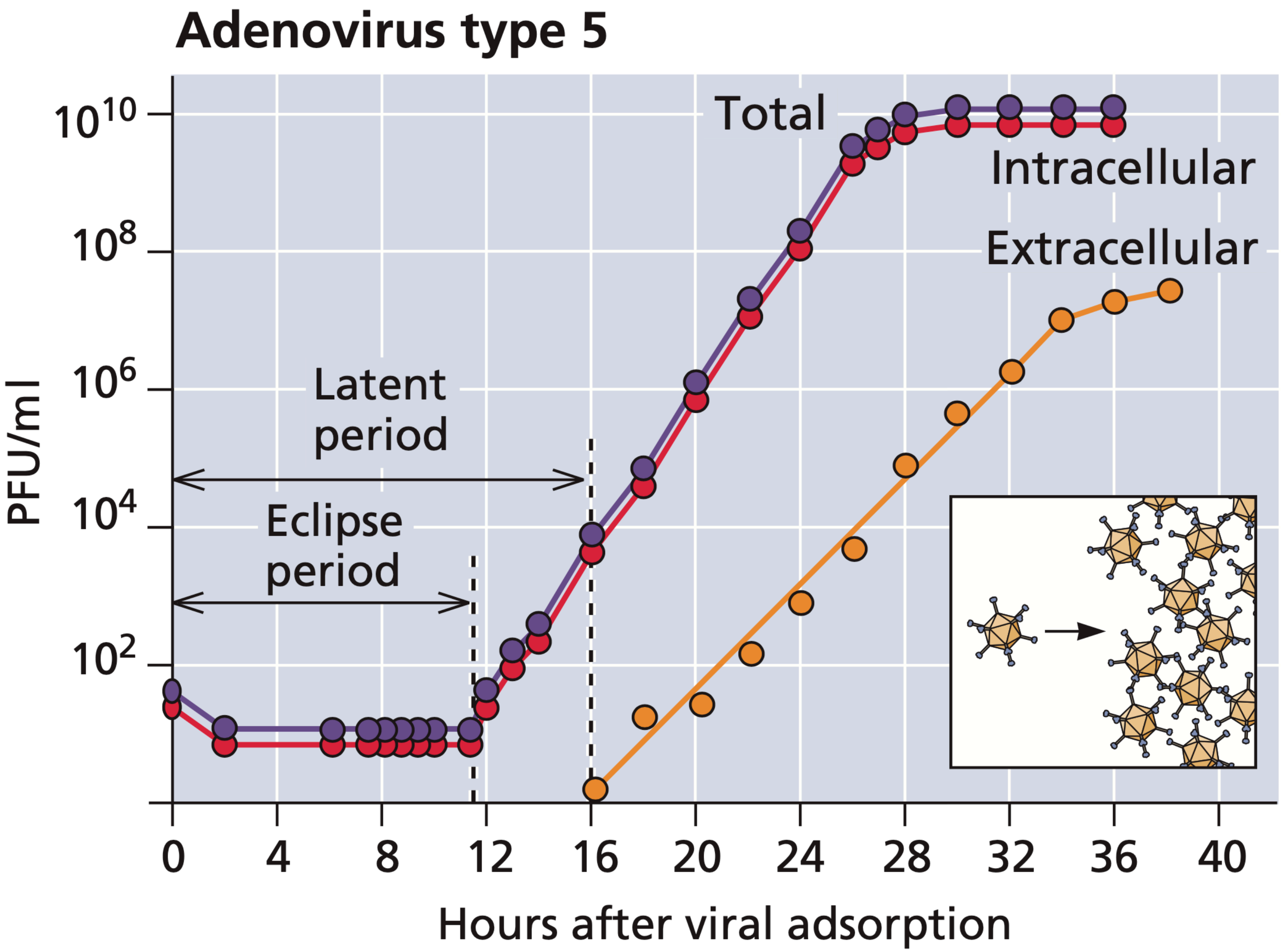
- Ellis & Delbruck, 1939, studies on *E. coli* bacteriophages
- Adsorb
- Dilute culture
- Sample
- Assay



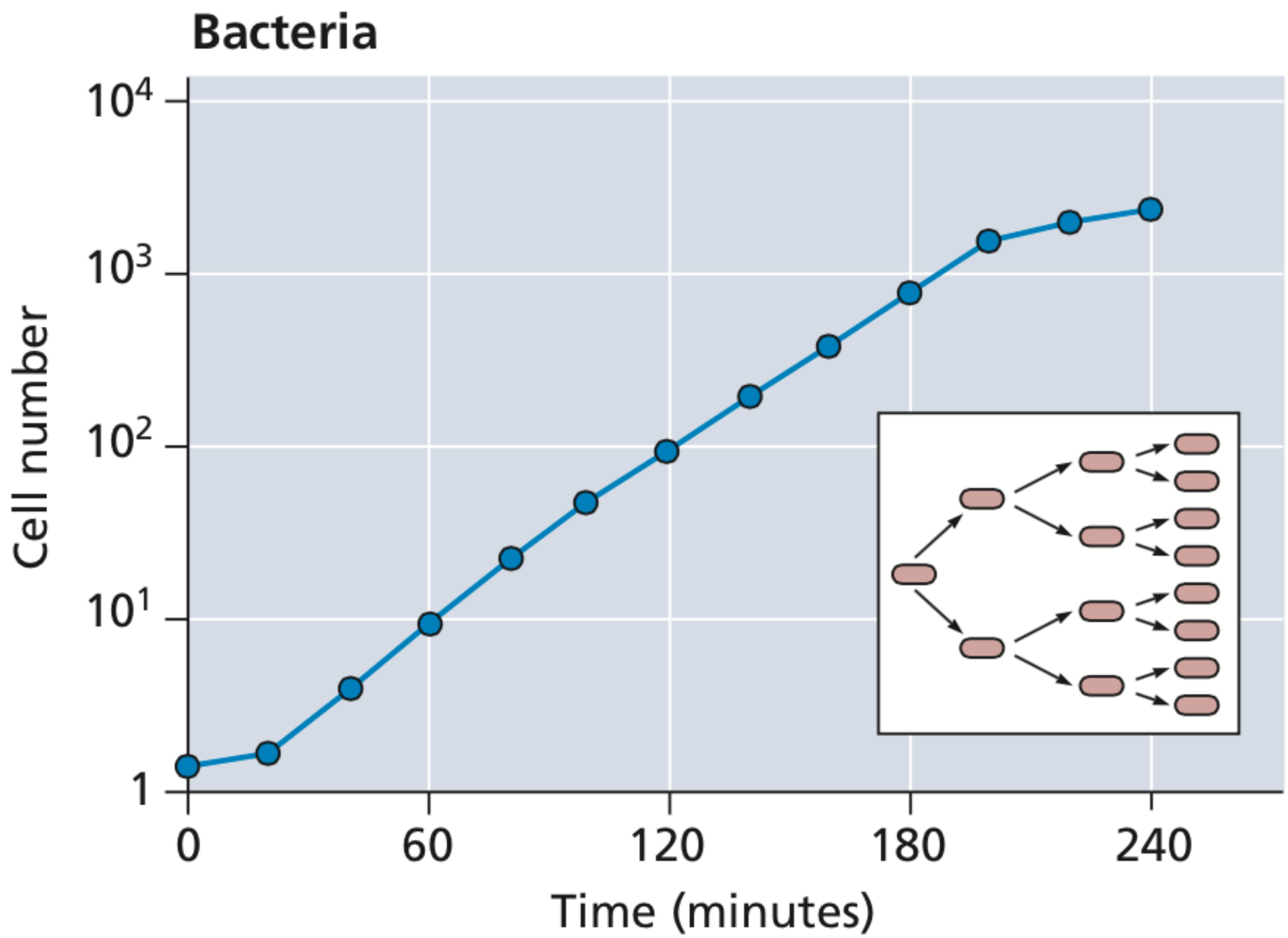
Single and multi-step growth cycles





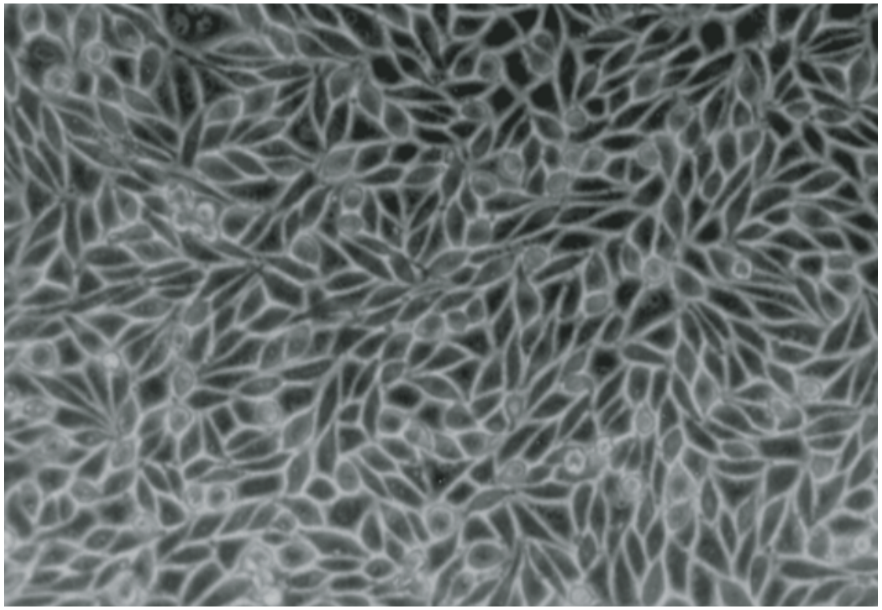


Adenovirus type 5
Total
Intracellular
Extracellular
Latent period
Eclipse period
PFU/ml
10^10
10^8
10^6
10^4
10^2
0
4
8
12
16
20
24
28
32
36
40
Hours after viral adsorption


Bacteria
10^4
10^3
10^2
10^1
1
Cell number
0
60
120
180
240
Time (minutes)

Synchronous infection - key to one-step growth cycle

To achieve this, we need to infect all the cells - but how do we know?

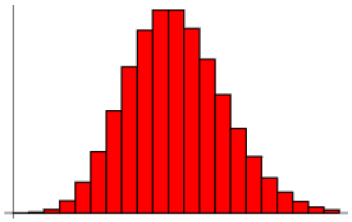


Multiplicity of infection (MOI)

- Number of infectious particles ADDED per cell
- Amount of virus (PFU) ÷ # of cells
- Not the number of infectious particles each cell *receives*
- Add 10^7 virus particles to 10^6 cells - MOI of 10 - each cell does NOT receive 10 virus particles

MOI

- Infection depends on the random collision of virus particles and cells
- When susceptible cells are mixed with virus, some cells are uninfected, some receive one, two, three or more particles
- The distribution of virus particles per cell is best described by the *Poisson distribution*



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

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

m: multiplicity of infection (moi)

uninfected cells: $P(0) = e^{-m}$

cells receiving 1 particle: $P(1) = me^{-m}$

cells multiply infected: $P(>1) = 1-e^{-m}(m+1)$

[obtained by subtracting from 1 {the sum of all probabilities for any value of k} the probabilities *P(0)* and *P(1)*]

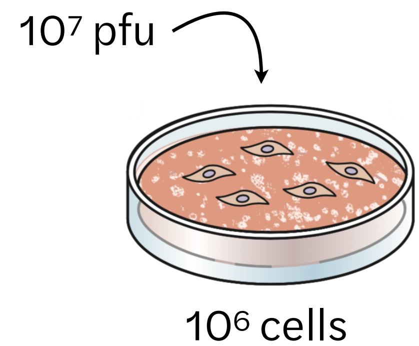
Examples:

If 10^6 cells are infected at **moi of 10**:

45 cells are uninfected

450 cells receive 1 particle

the rest receive >1 particle

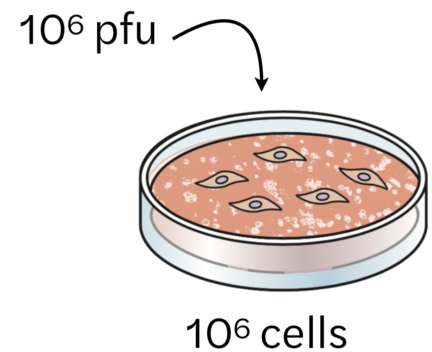


If 10^6 cells are infected at **moi of 1**:

37% of the cells are uninfected

37% of the cells receive 1 particle

26% receive >1 particle

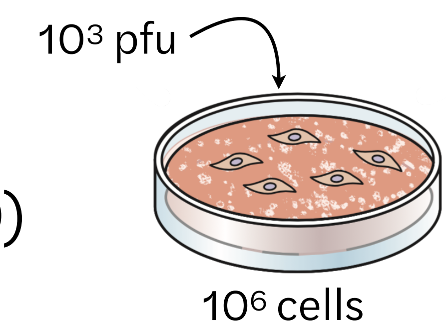


If 10^6 cells are infected at **moi of .001**:

99.9% of the cells are uninfected

00.099% of the cells receive 1 particle (990)

00.0001% receive >1 particle



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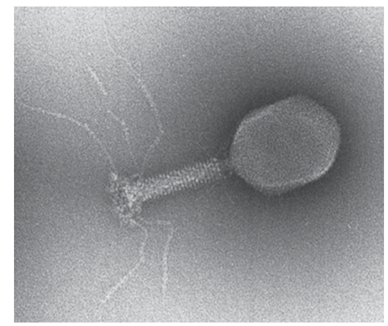
If cells are infected at an MOI=10 in a one-step growth cycle experiment, in the growth curve you will likely see…

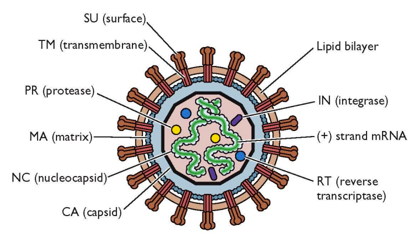
A. Multiple bursts of virus release
B. Multiple eclipse periods
C. A single burst of virus release
D. No burst of virus release
E. Asynchronous infection

Physical measurements of virus particles

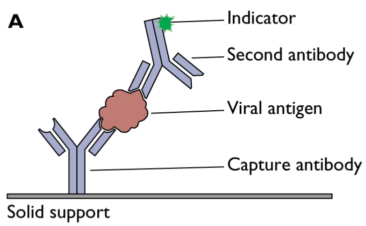
- Hemagglutination

- Electron microscopy

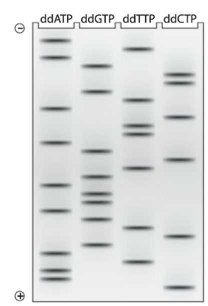




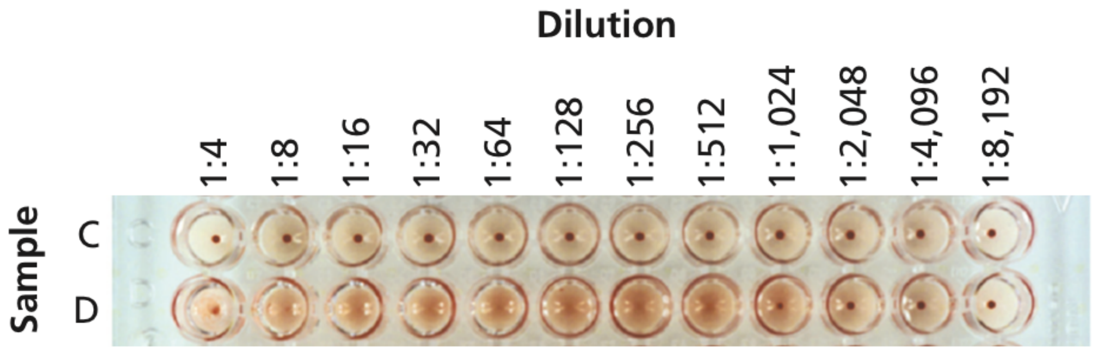
- Viral enzymes

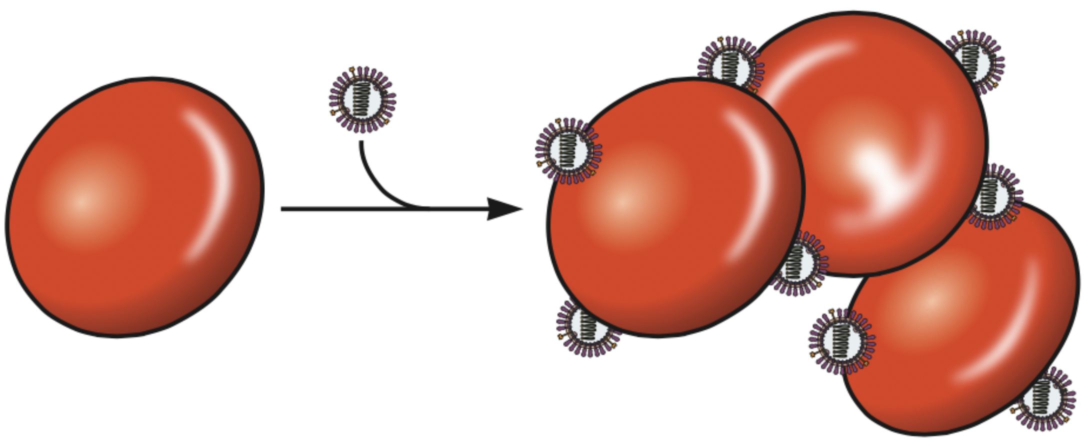


- Serology

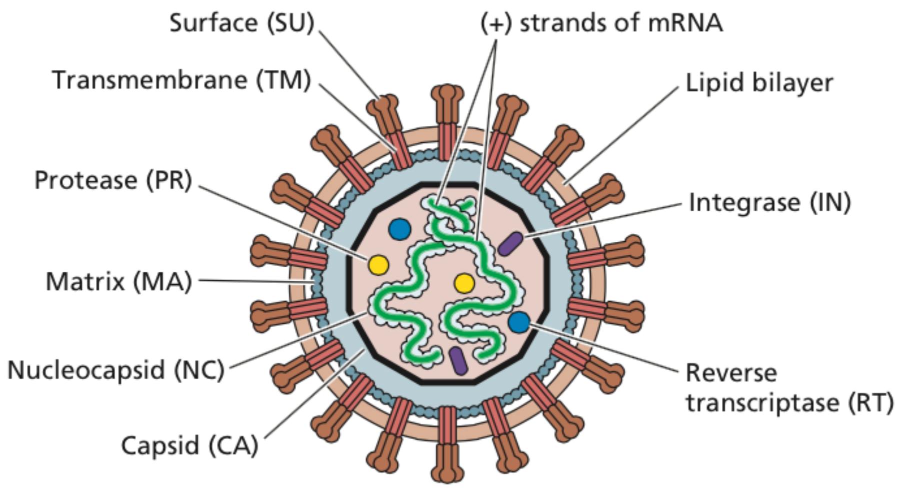


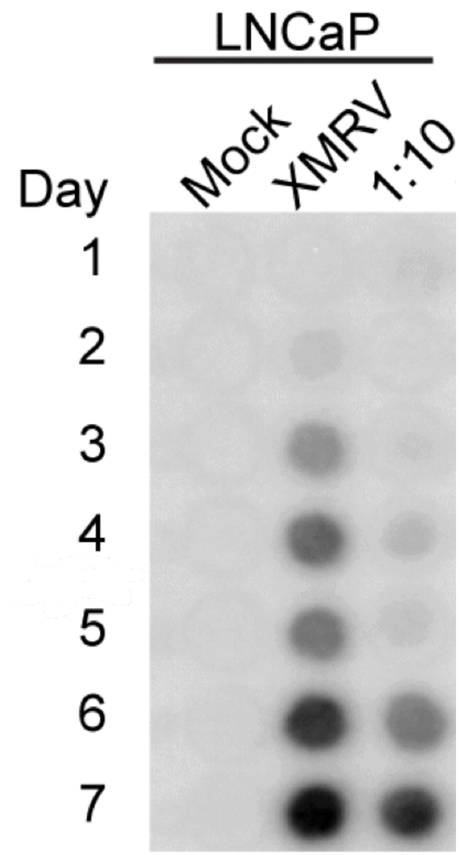
Hemagglutination



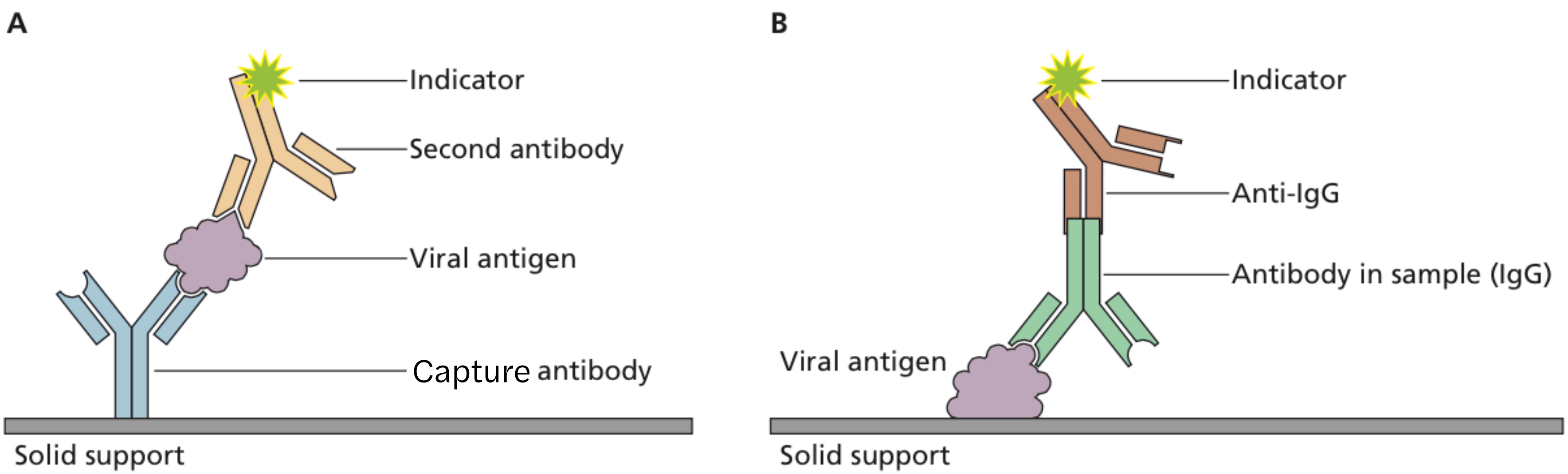


Measurement of viral enzyme activity

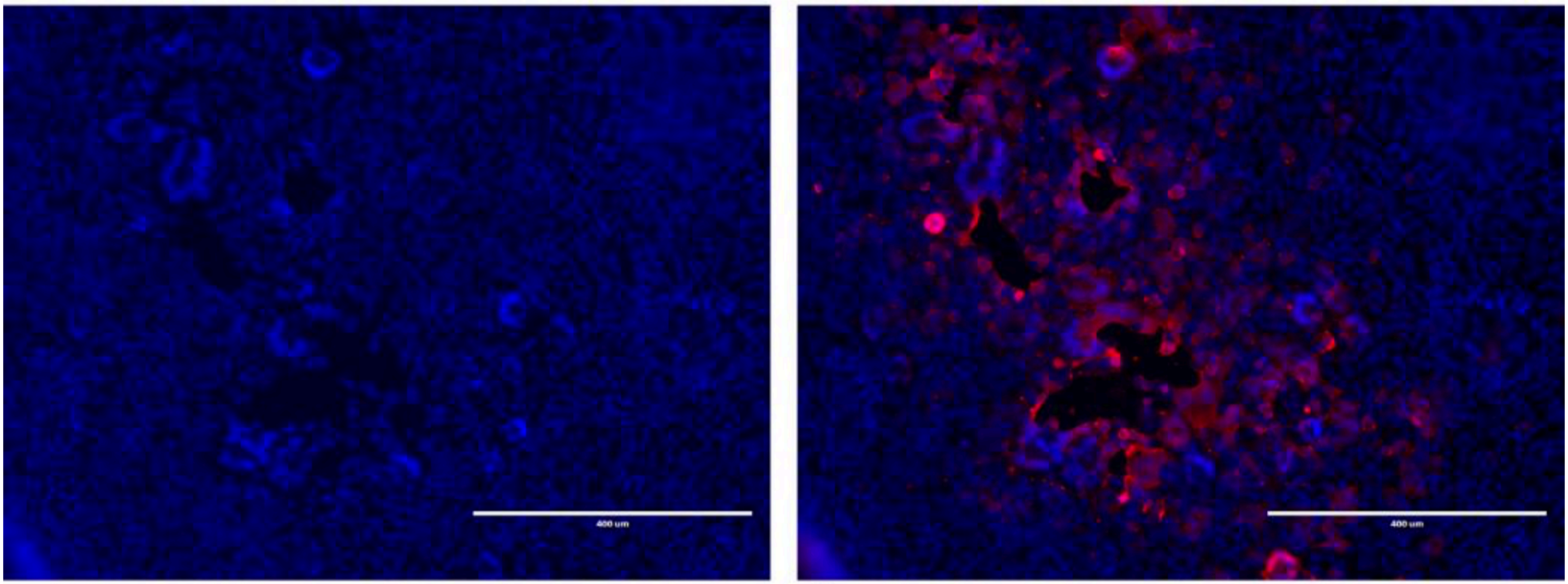




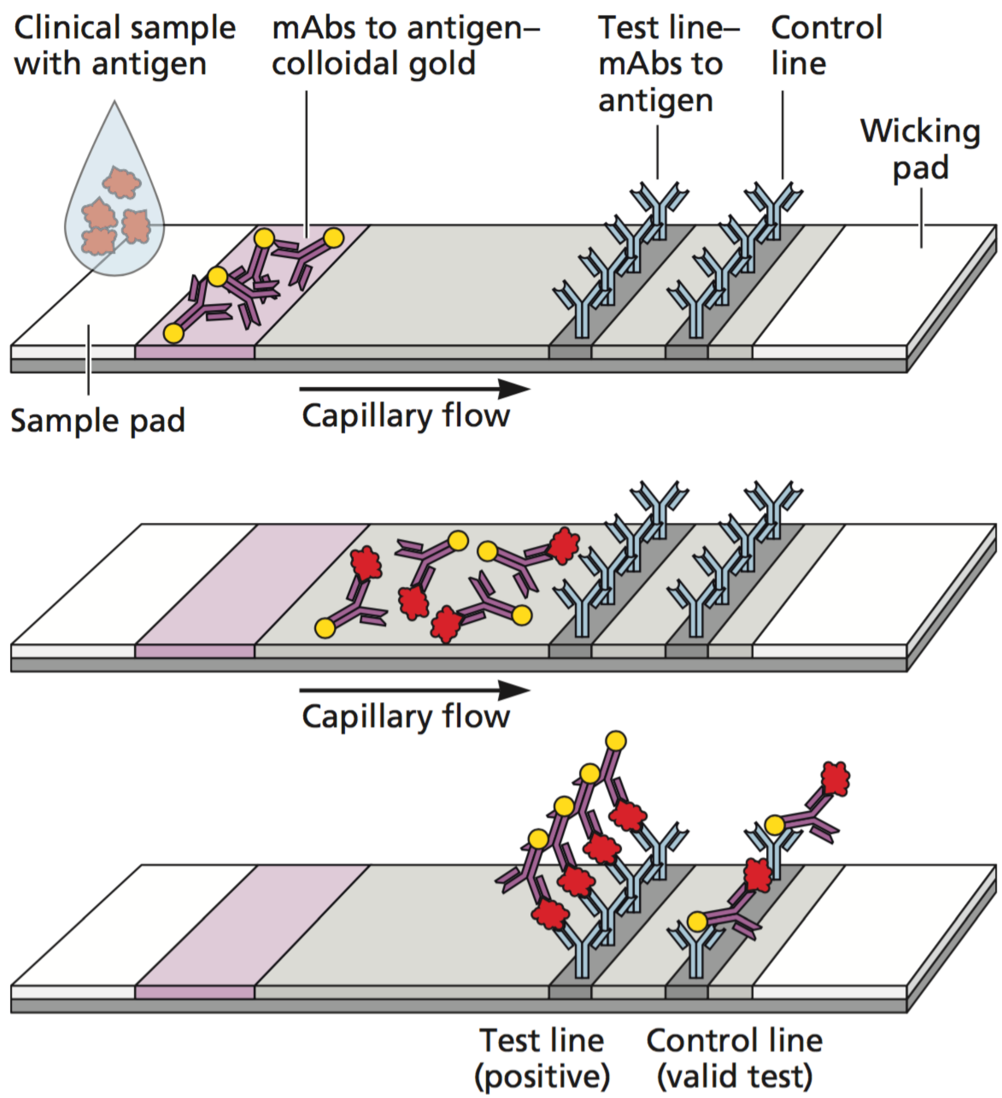
Enzyme-linked immunosorbent assay (ELISA): detecting viral antigens or antibodies



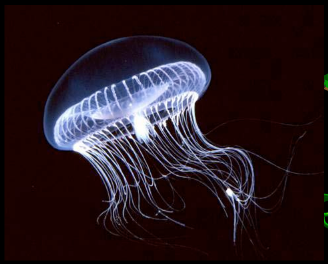
incubation (Extended Data Figure 5a and 5b). The identity of the strain WIV04 was verified in Vero E6 cells by immunofluorescence microscopy using cross-reactive viral NP antibody (Extended Data Figure 5c and 5d), and by metagenomic sequencing,

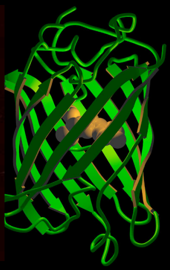


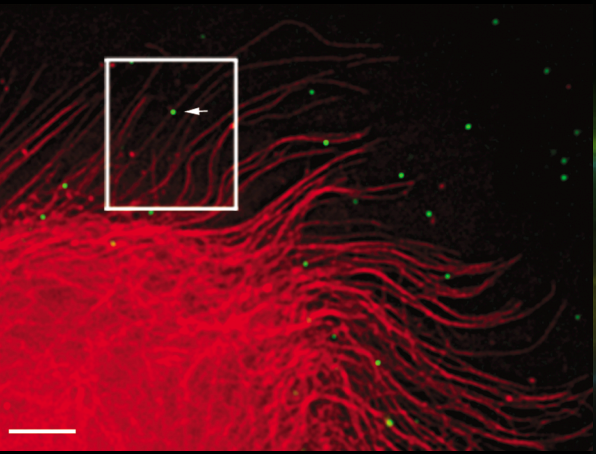
https://doi.org/10.1038/s41586-020-2012-7

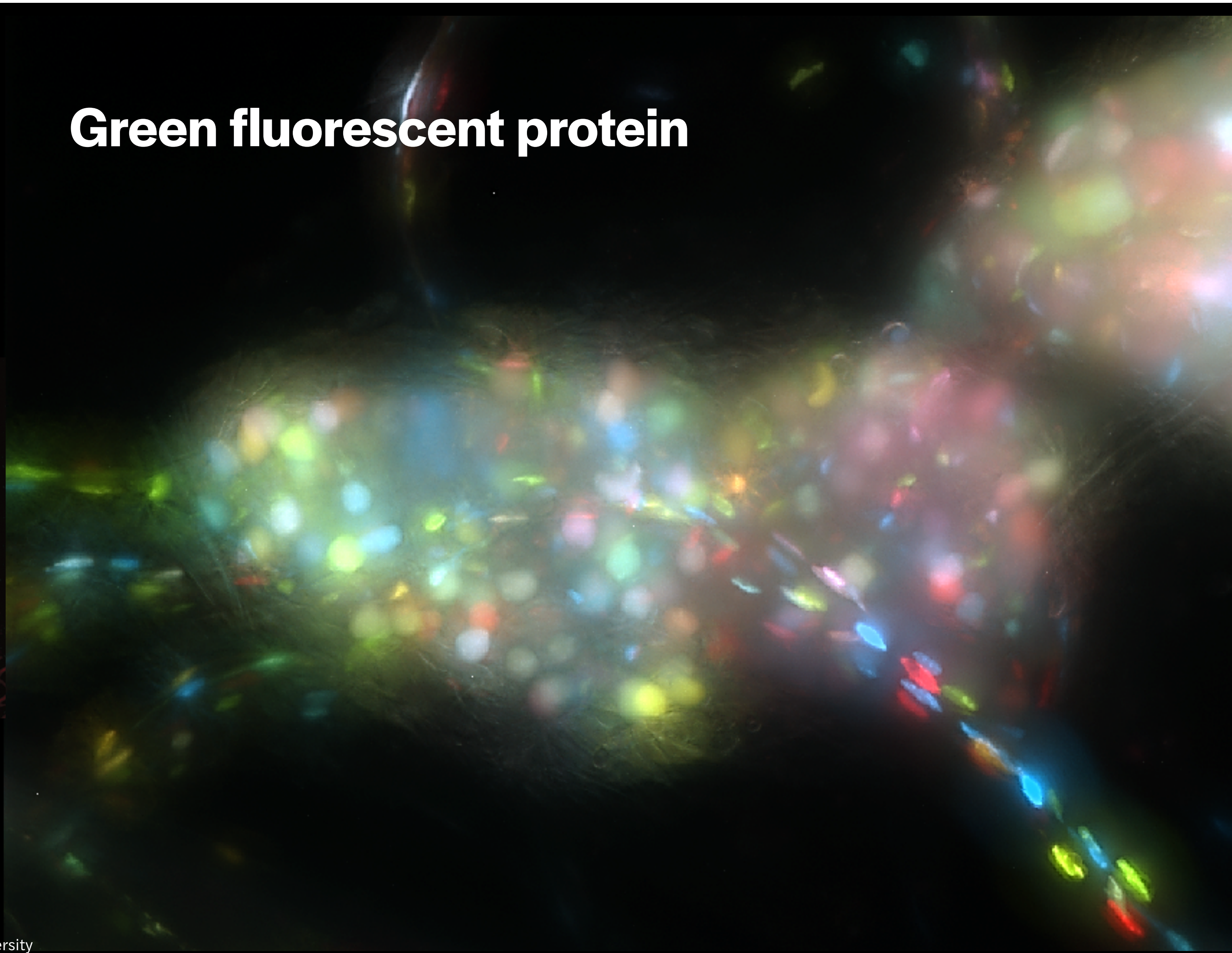

Clinical sample with antigen
mAbs to antigen–colloidal gold
Test line–mAbs to antigen
Control line
Wicking pad
Sample pad
Capillary flow
Capillary flow
Test line (positive)
Control line (valid test)

Green fluorescent protein









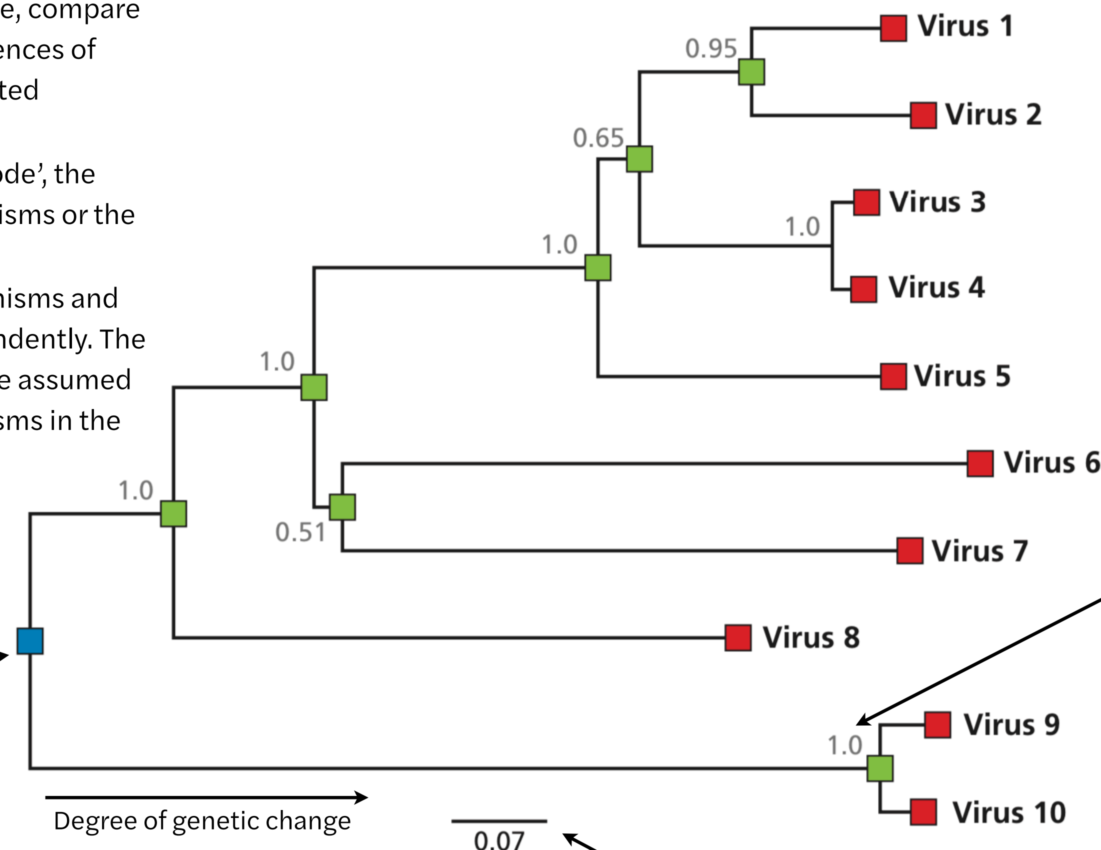
Deep, high-throughput sequencing

- Metagenomics
- Identification of new viruses in a sample
- Identification of new pathogens
- Human genome: 10 yr/$3B vs 1 day/$1000

(this is not DHTS)

Phylogenetic trees

- Phylogenetic trees measure the genetic distance between organisms, and identify the nearest relatives
- To construct a phylogenetic tree, compare differences in nucleotide sequences of many isolates of putatively related organisms
- Each division in the tree is a ‘node’, the common ancestor of the organisms or the isolates identified to its right
- After such branching, the organisms and their sequences evolve independently. The ‘root’ (at the extreme left) is the assumed common ancestor of all organisms in the tree



Measure of support: Sequences to right of node cluster together better than other sequences

Root = presumed ancestor

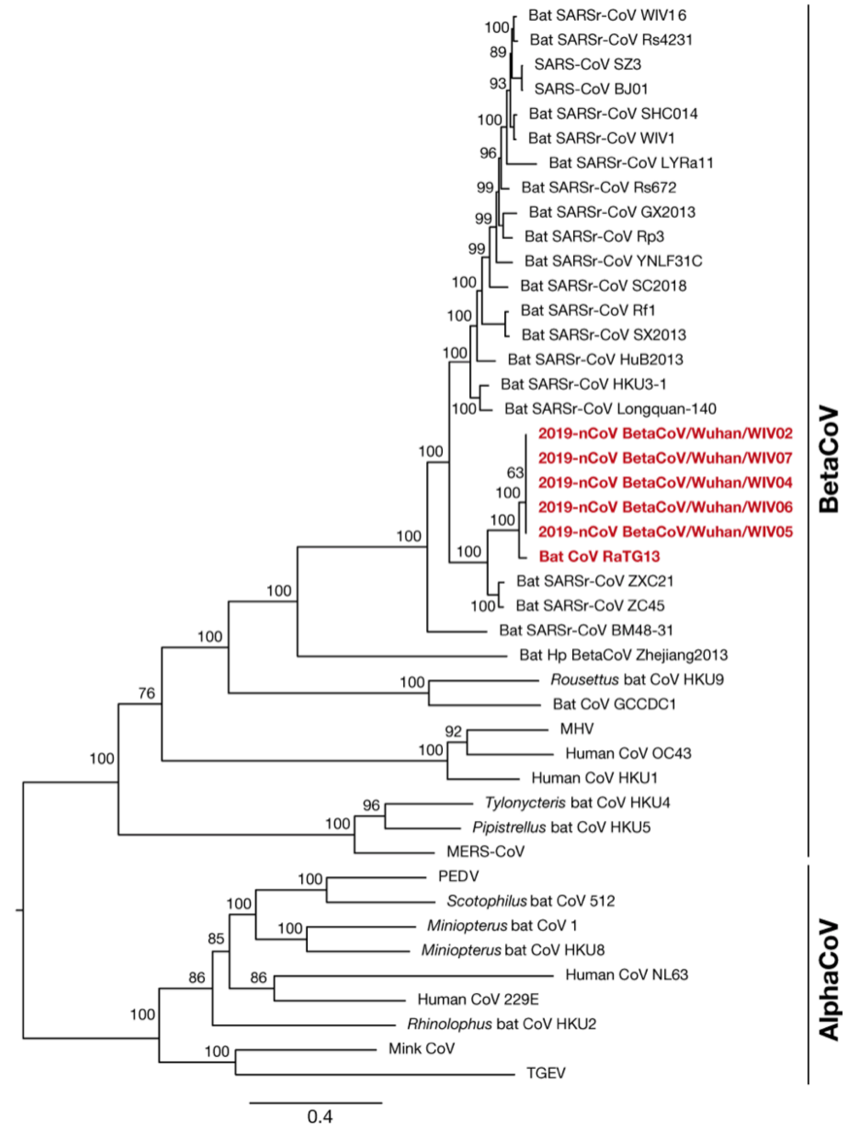
Scale = # changes/length of sequence

Article | Open Access | Published: 03 February 2020

A pneumonia outbreak associated with a new coronavirus of probable bat origin

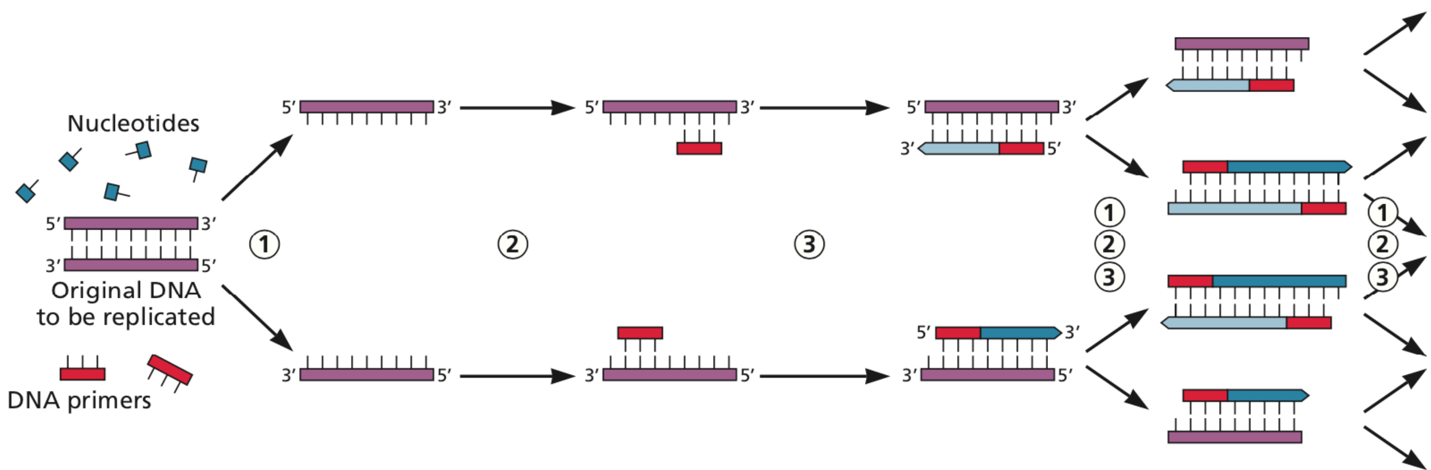
Peng Zhou, Xing-Lou Yang, [...] Zheng-Li Shi

Nature **579**, 270–273(2020) | Cite this article

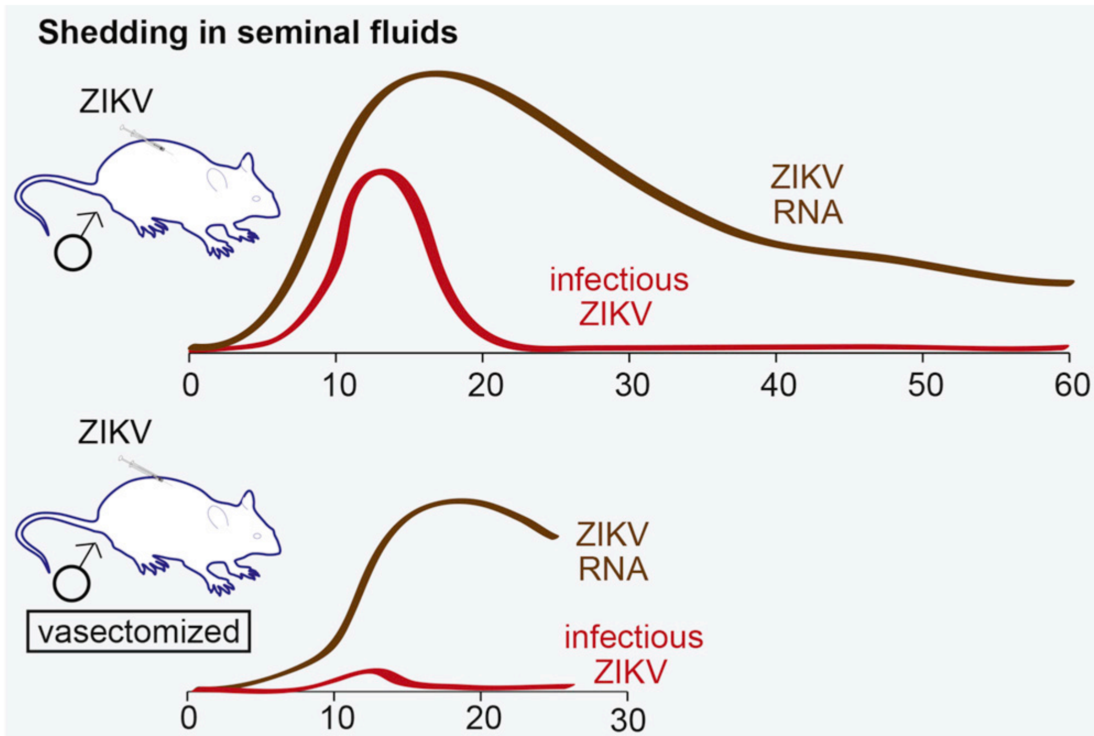


Polymerase chain reaction (PCR)

- Research
- Industry
- Diagnosis



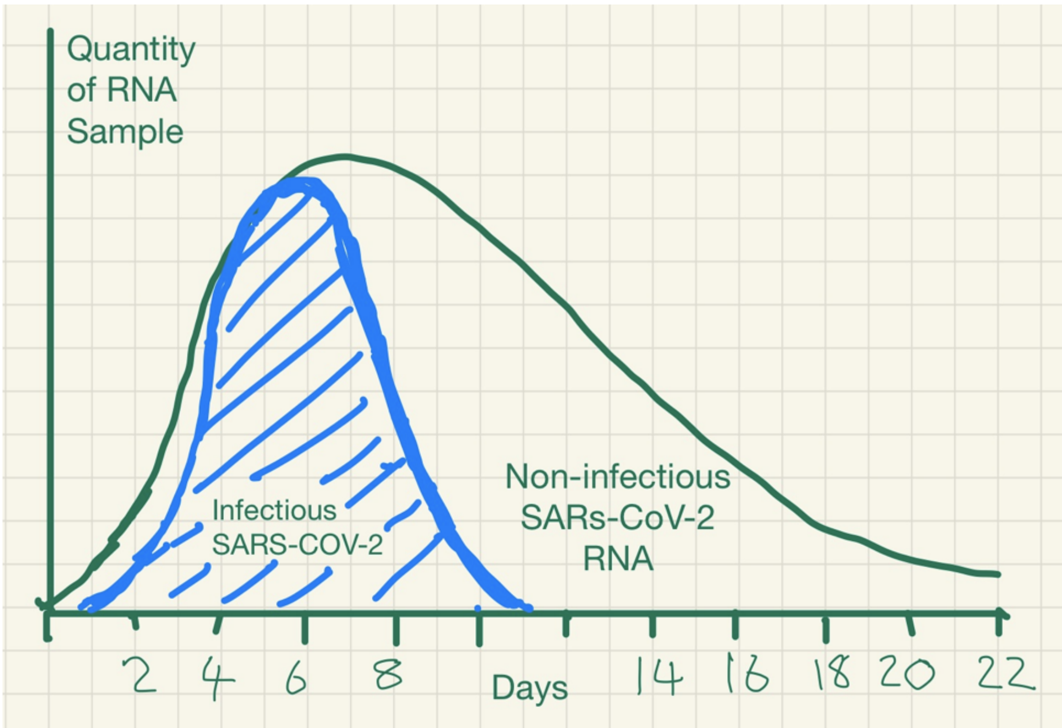
PCR product is not the same as infectious virus



For many RNA viruses, RNA can be detected long after disappearance of infectious virus

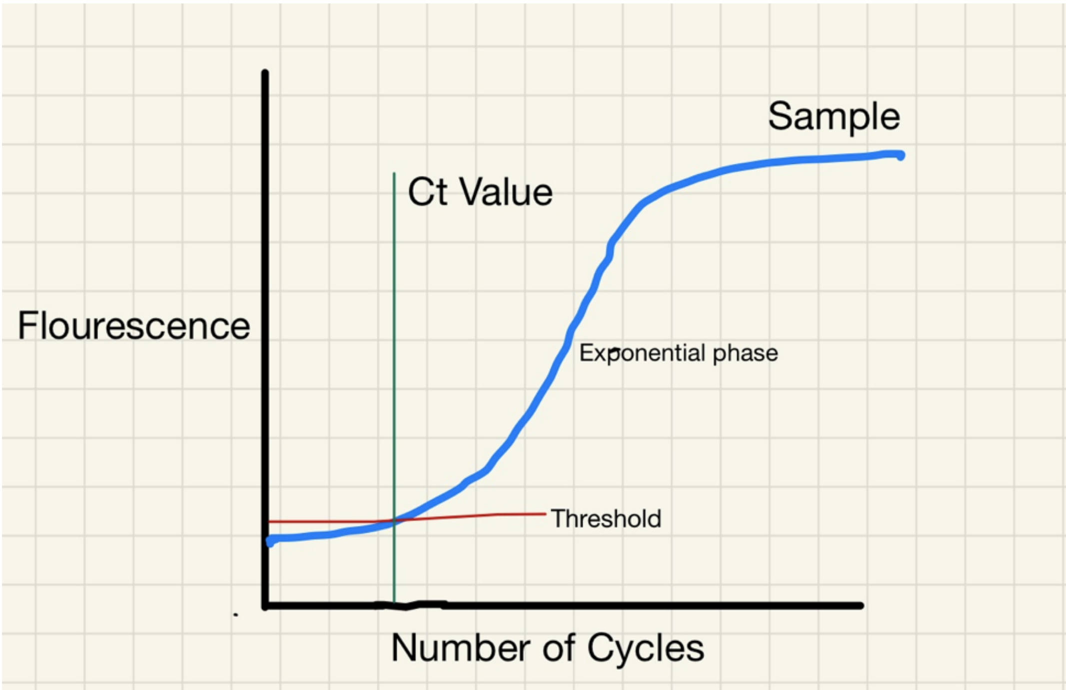
https://doi.org/10.1016/j.celrep.2017.01.056

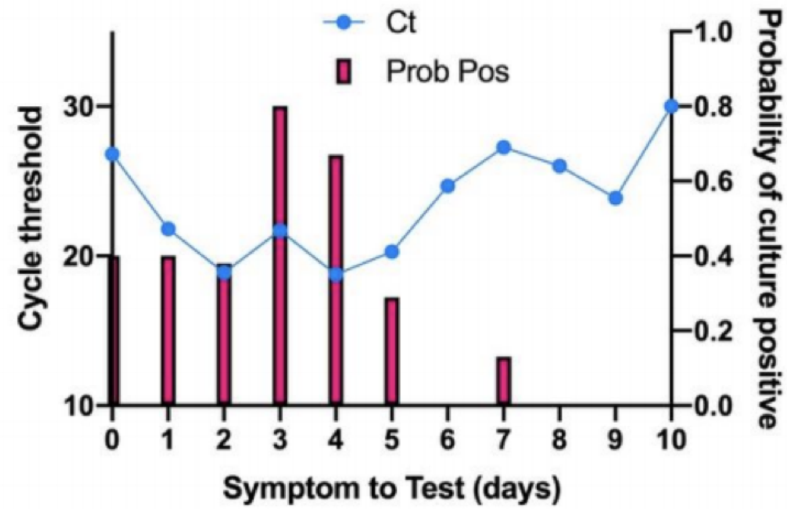
SARS-CoV-2 RNA and infectivity



https://www.cebm.net/covid-19/infectious-positive-pcr-test-result-covid-19/

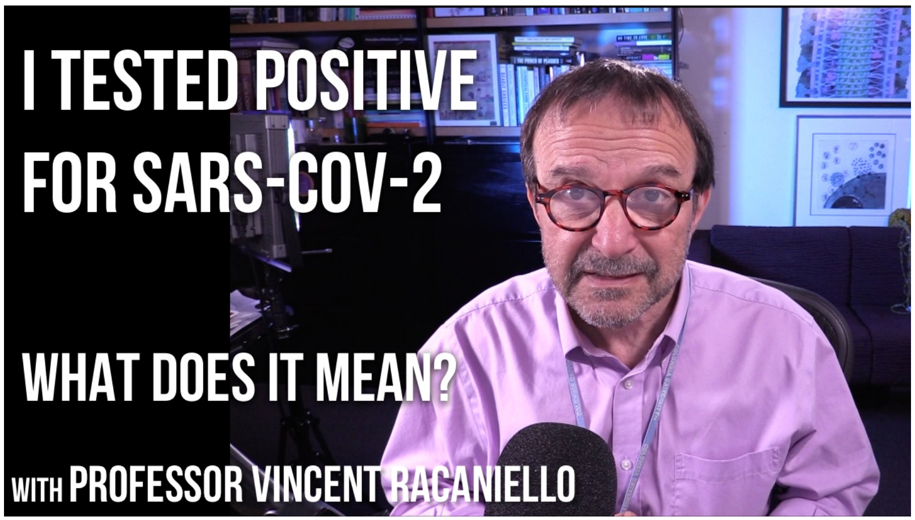
Cycle threshold and SARS-CoV-2





https://www.cebm.net/covid-19/infectious-positive-pcr-test-result-covid-19/

My experience with Ct and antibody tests



https://youtu.be/Lk64Zwcj3W8

https://youtu.be/HvXCISbrK9Q

VIROLOGY LIVE

WITH VINCENT RACANIELLO

Next time: Genomes and Genetics