The Infectious Cycle

Lecture 2

Biology W3310/4310

Virology

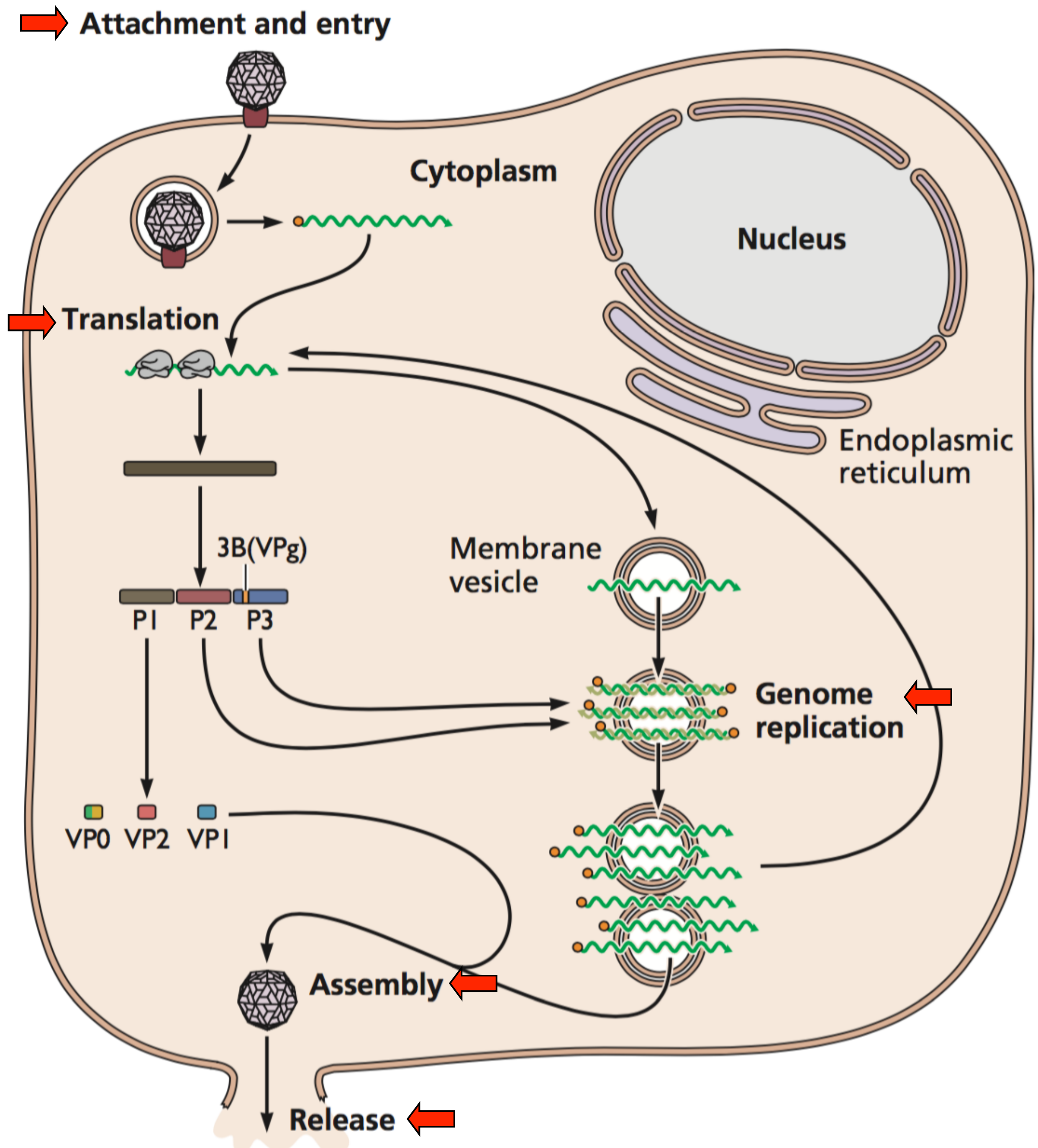
Spring 2016

"You know my methods, Watson"

--Sir Arthur Conan Doyle

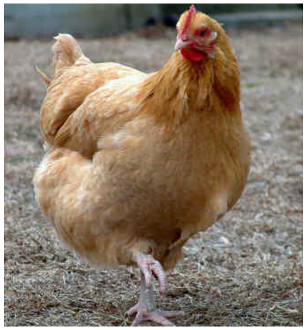
The Infectious Cycle

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



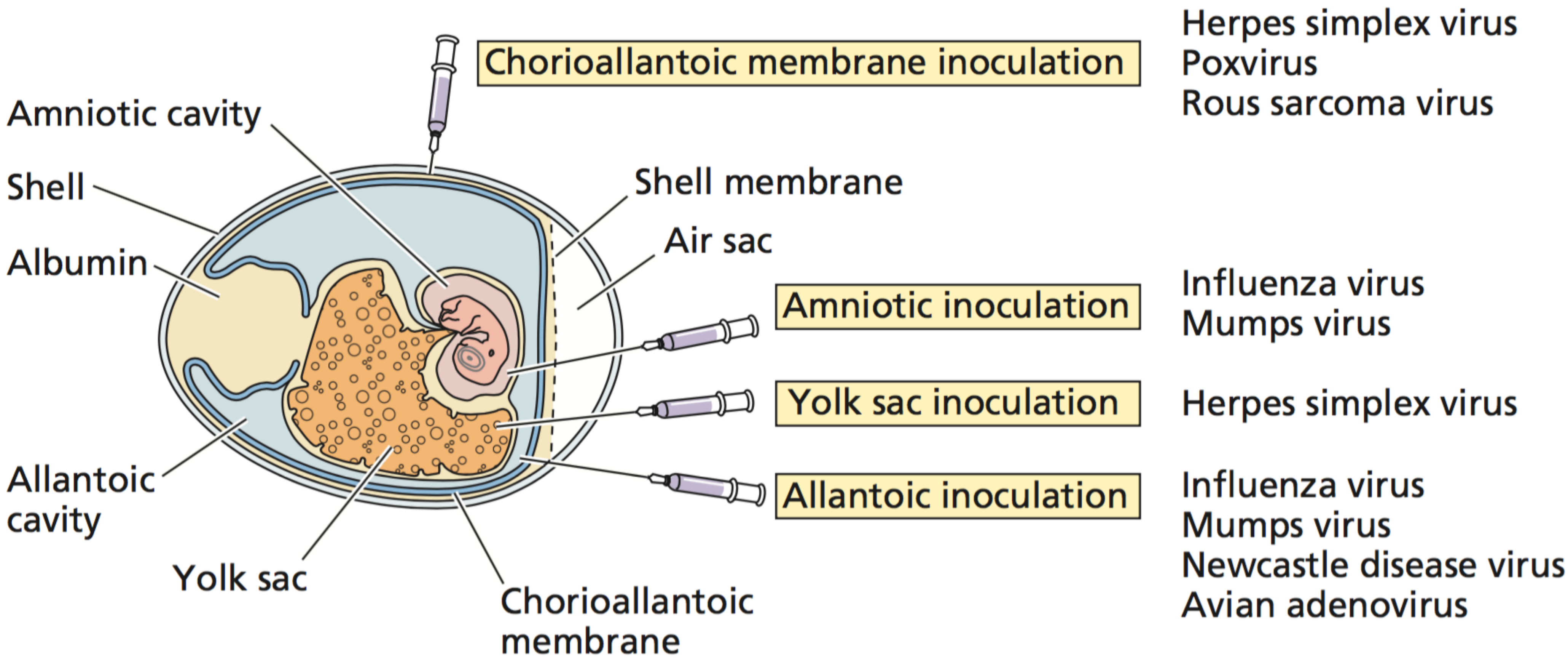
Some important definitions

- 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



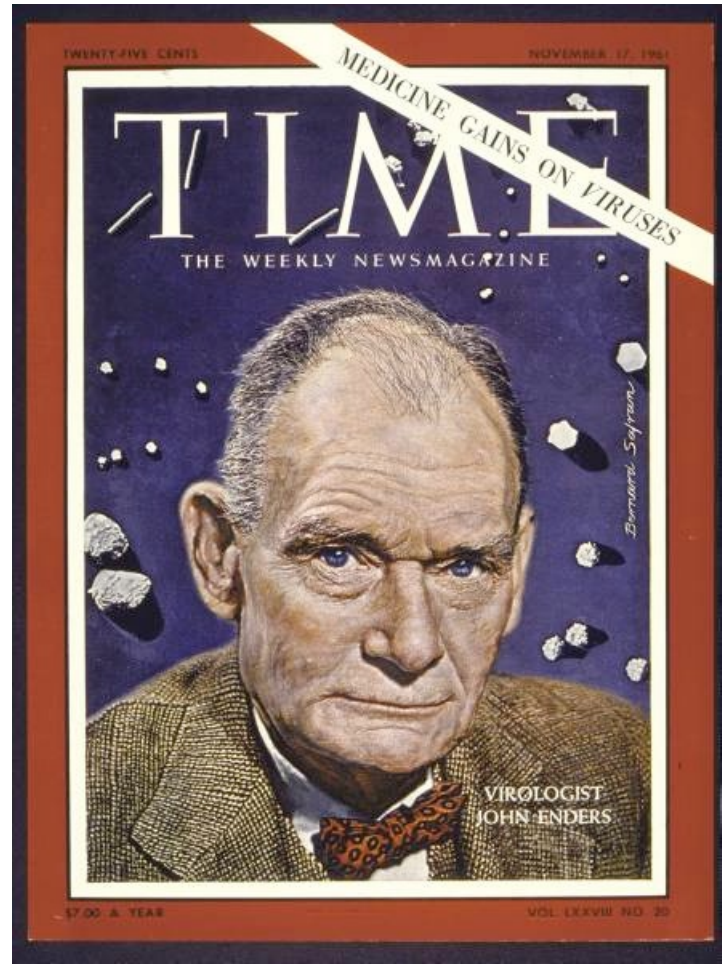

Chorioallantoic membrane inoculation
Herpes simplex virus
Poxvirus
Rous sarcoma virus
Amniotic cavity
Shell
Shell membrane
Air sac
Albumin
Amniotic inoculation
Influenza virus
Mumps virus
Yolk sac inoculation
Herpes simplex virus
Allantoic cavity
Allantoic inoculation
Influenza virus
Mumps virus
Newcastle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


Embryonated eggs at 10 to 12 days being inoculated by automated machinery. 1st larger needle (about 1 mm diameter) punches a hole in a shell and 2nd smaller needle injects a seed into the allantoic cavity of the egg followed by incubation for 2 to 3 days. It takes less than 10 seconds to inoculate a row of eggs.
Courtesy: Solvay

ifpma.org

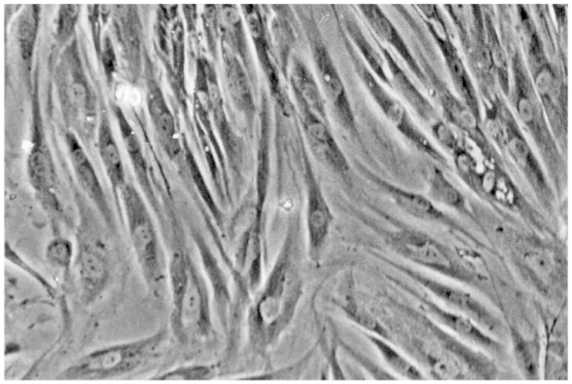
Studying the infectious cycle in cells

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

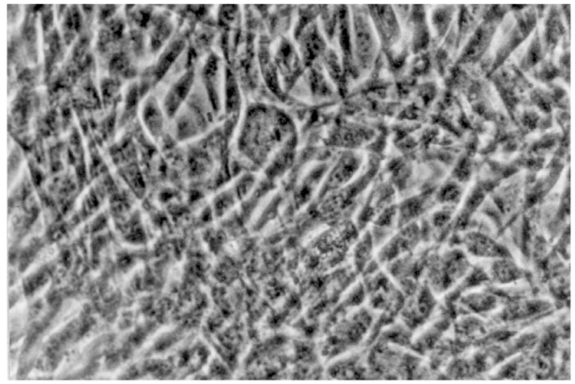


Virus cultivation

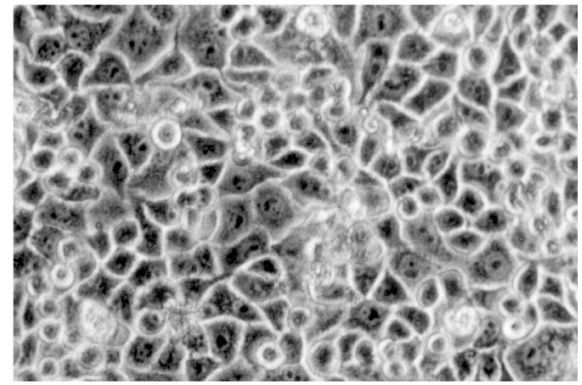
A



B



C



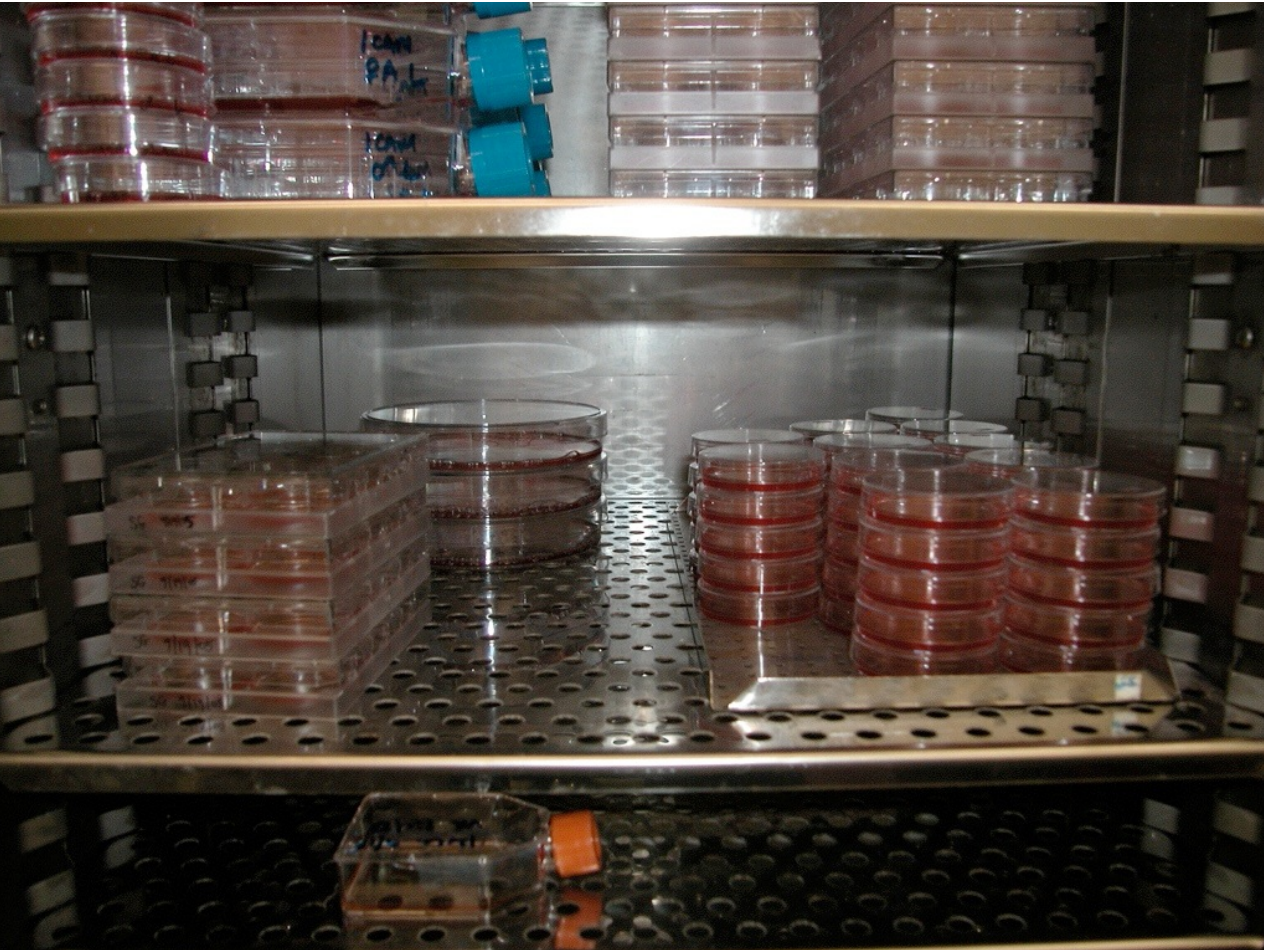
primary human foreskin fibroblasts

mouse fibroblast cell line (3T3)

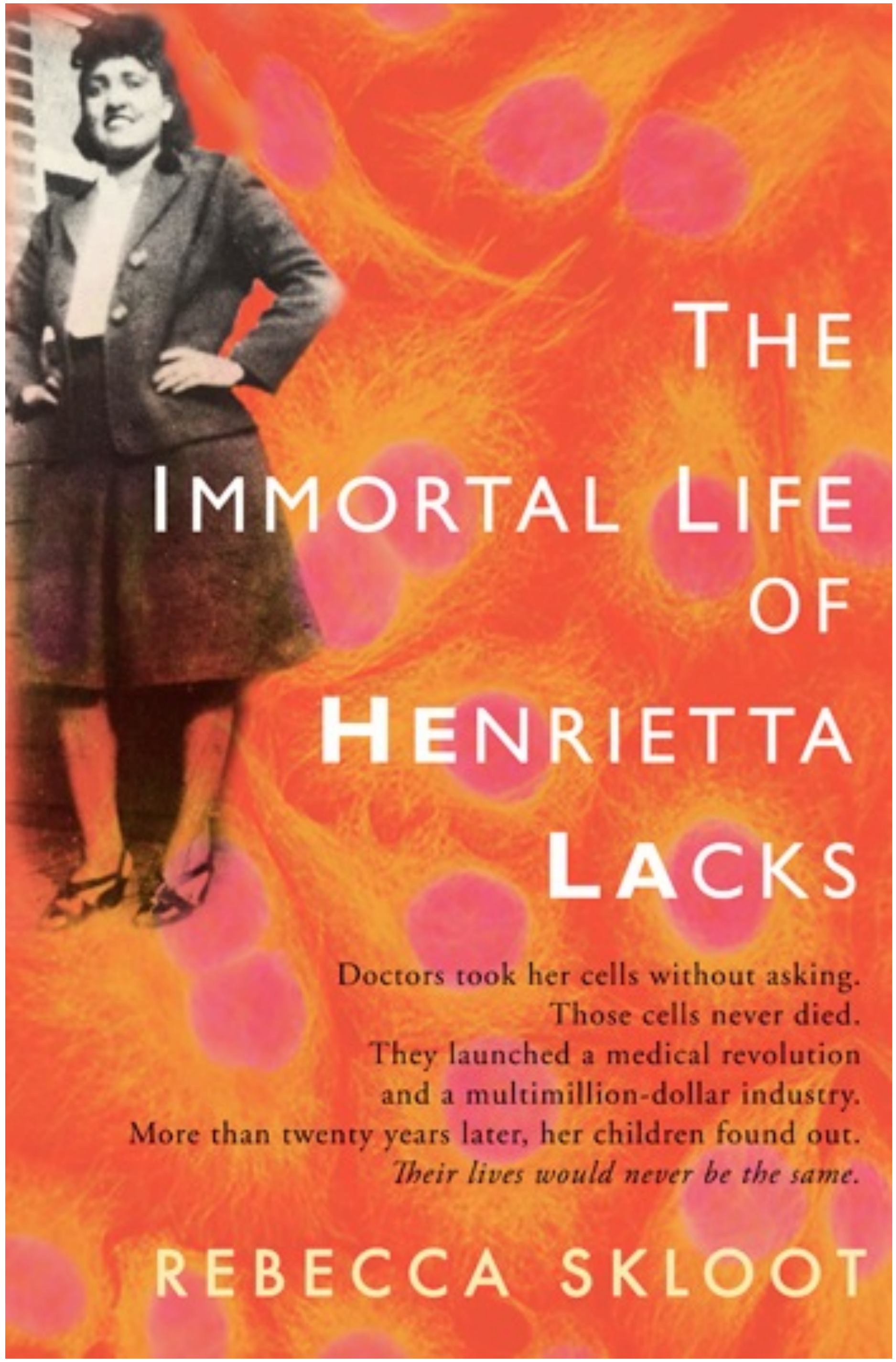
human epithelial cell line (HeLa)

continuous cell lines

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




9|9
μ-CARRIER
SPINNER FLASK
1965-01000
1000



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

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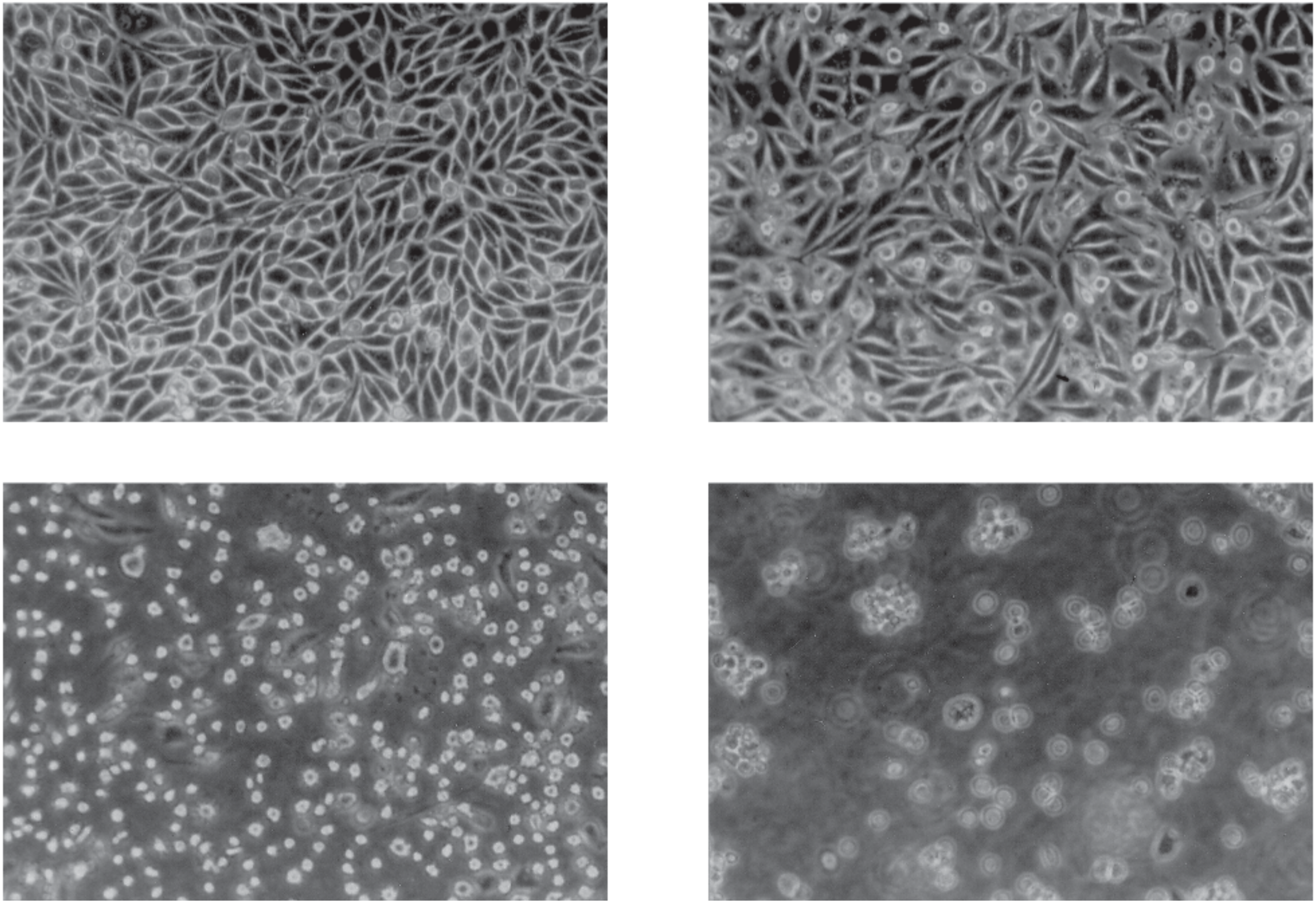
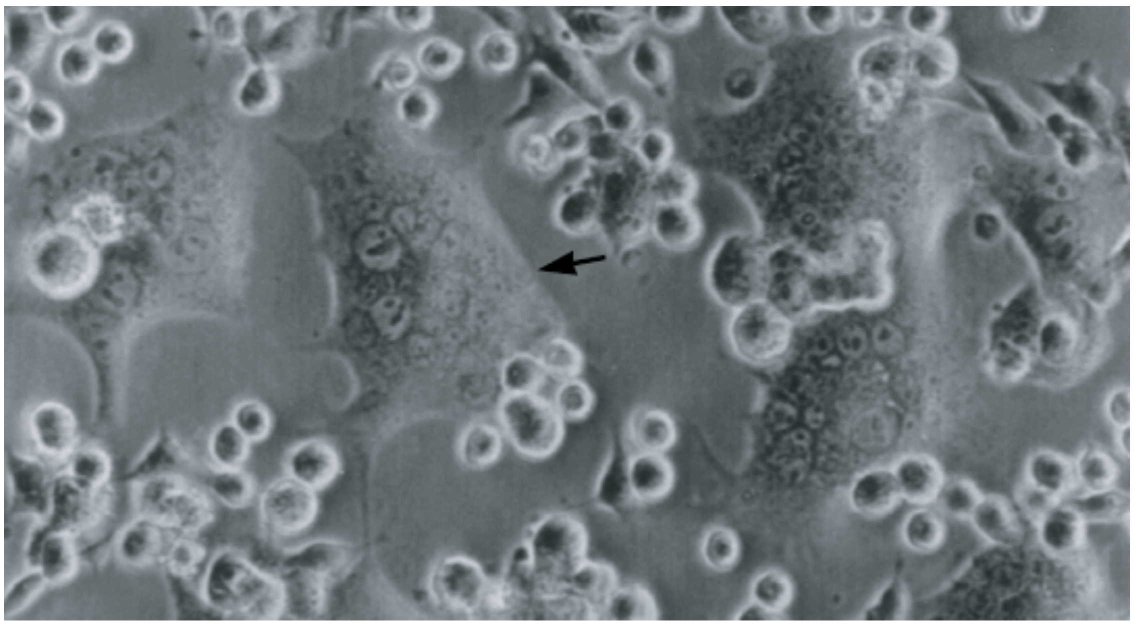
A _______ and _______ cell is the only cell that can take up a virus particle and replicate it (fill in the blanks)

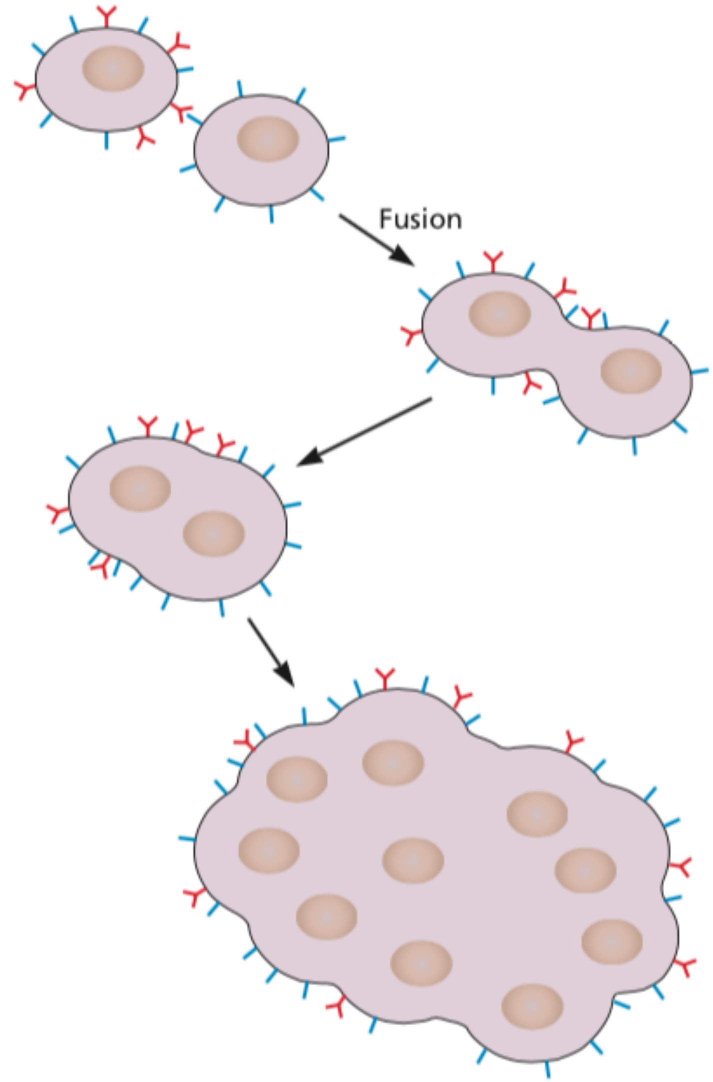
1. Naive and resistant
2. Primary and permissive
3. Susceptible and permissive
4. Susceptible and naive
5. Continuous and immortal



cytopathic effect (CPE)

Formation of syncytial





Examples of cytopathic effects

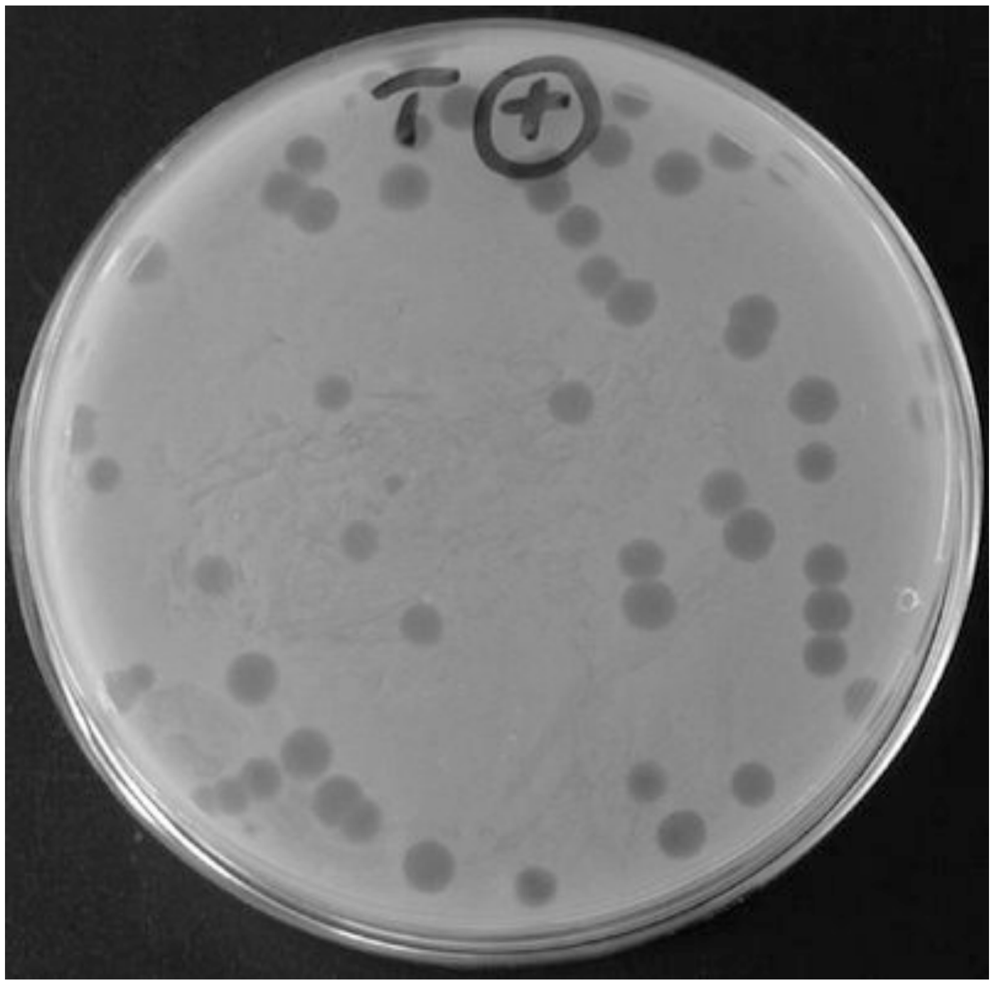
Cytopathic effect(s)	Virus(es)
Morphological alterations	
Nuclear shrinking (pyknosis), proliferation of membrane	Picornaviruses
Proliferation of nuclear membrane	Alphaviruses, herpesviruses
Vacuoles in cytoplasm	Polyomaviruses, papillomaviruses
Syncytium formation (cell fusion)	Paramyxoviruses, coronaviruses
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

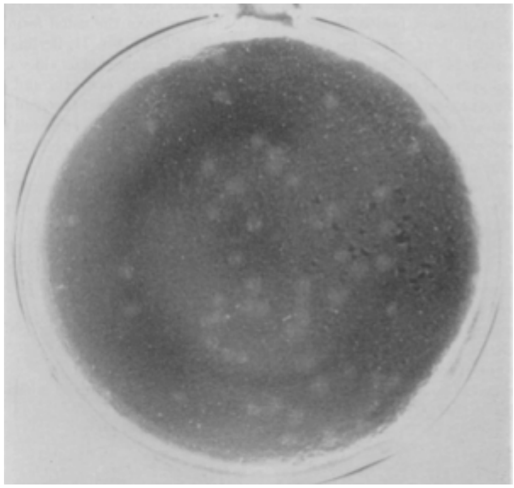
How many viruses in a sample?

- Infectivity
- Physical: virus particles and their components

Plaque assay

1930s: used to study multiplication of bacteriophages





Plaque assay

1952, Renato Dulbecco developed for animal viruses

Nobel Prize, 1975

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

BY RENATO DULBECCO

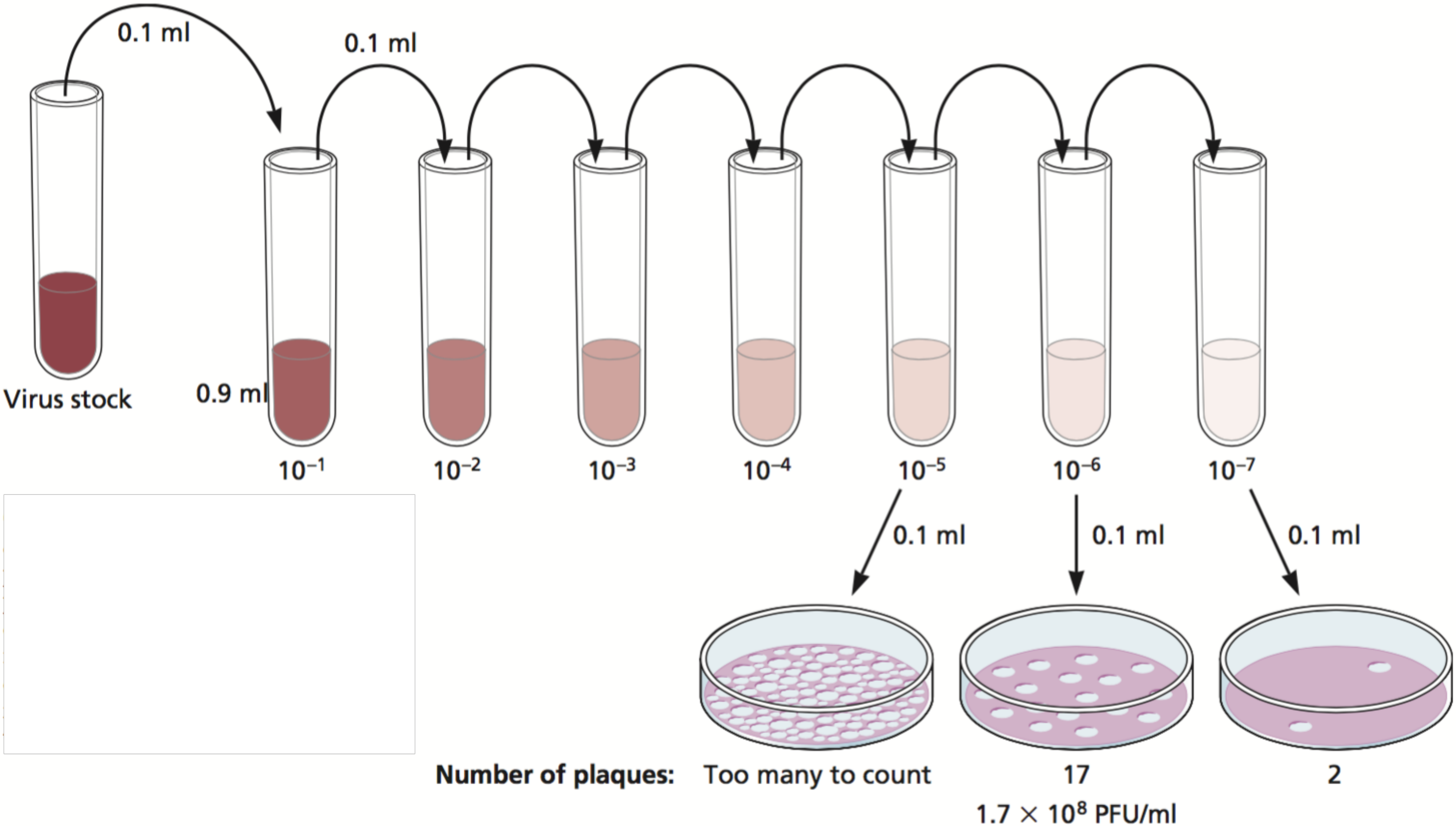
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

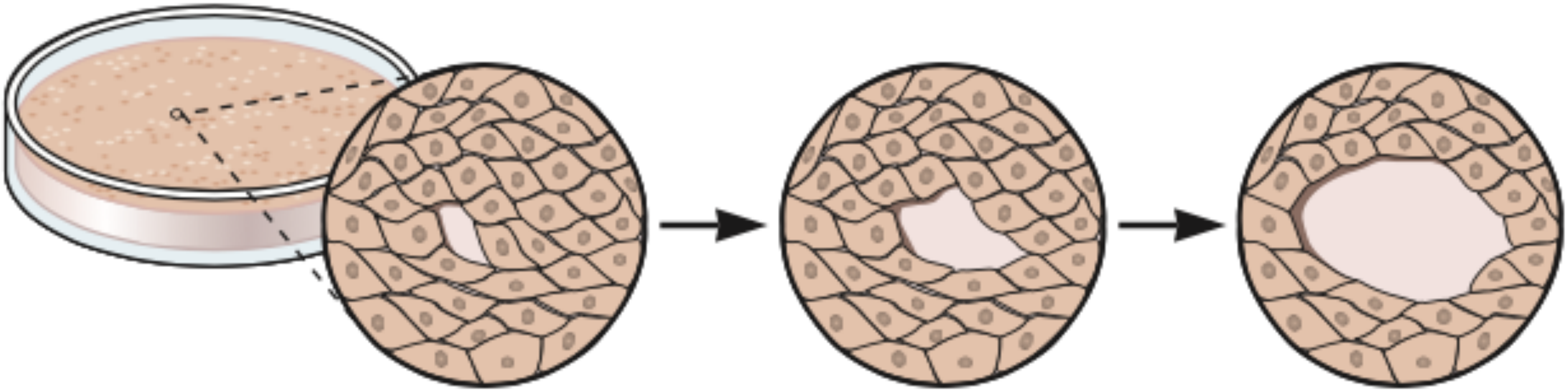
Read before the Academy, April 29, 1952

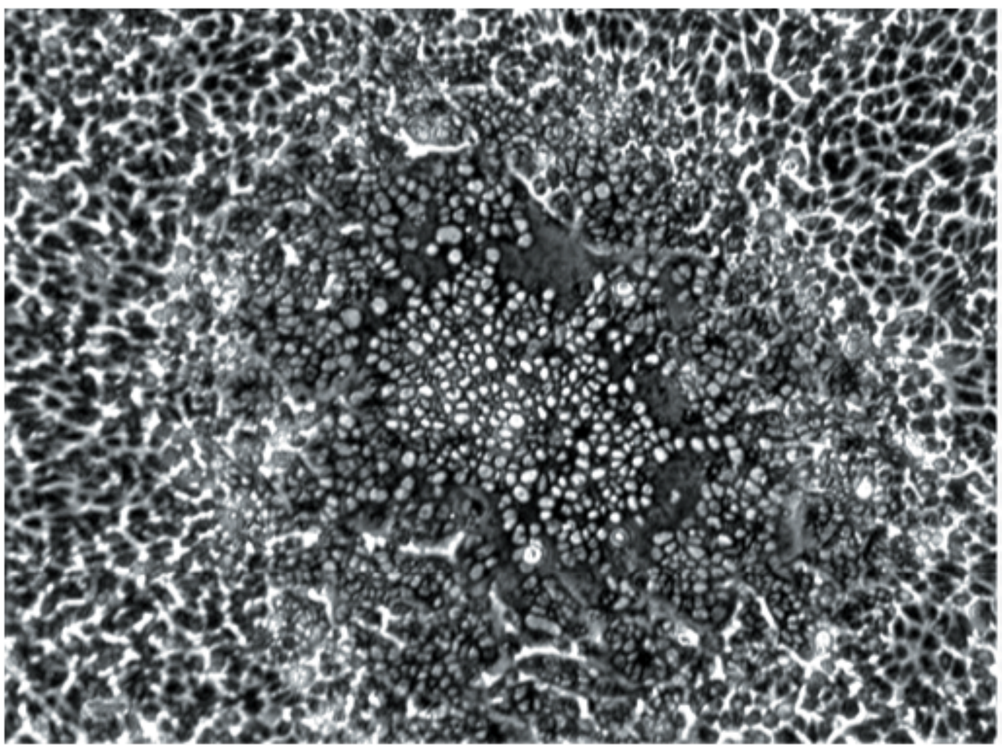
Research on the growth characteristics and genetic properties of animal viruses has stood greatly in need of improved quantitative techniques, such as those used in the related field of bacteriophage studies.

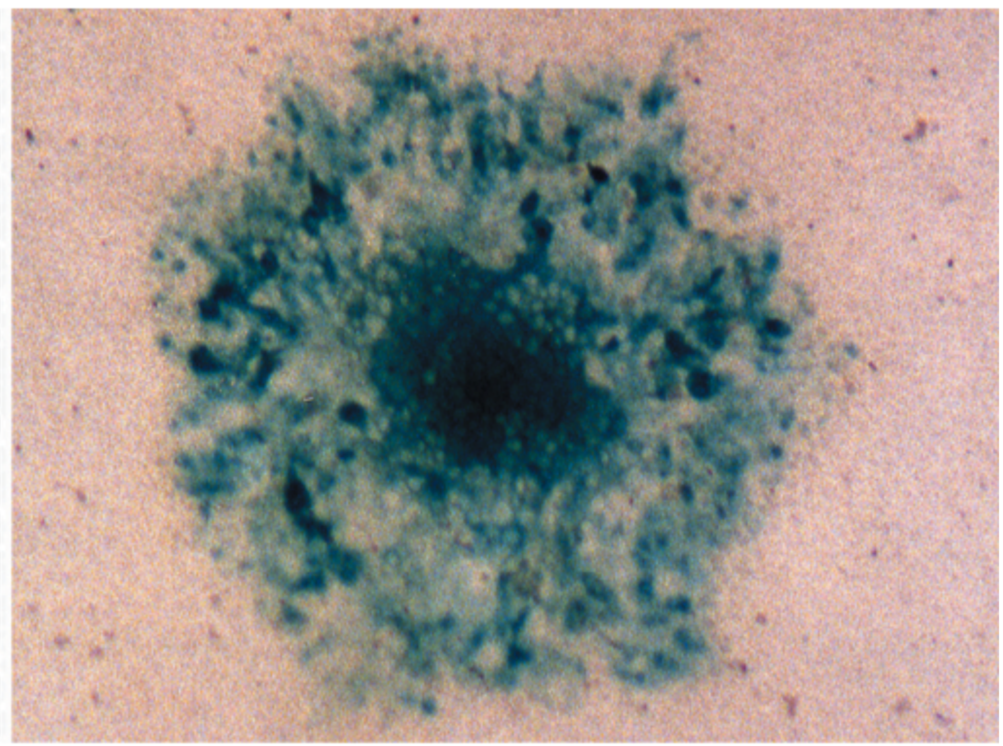
The requirements for a quantitative virus technique are as follows: (1) The use of a uniform type of host cell; (2) an accurate assay technique; (3) the isolation of the progeny of a single virus particle; and (4) the separate isolation of each of the virus particles produced by a single infected

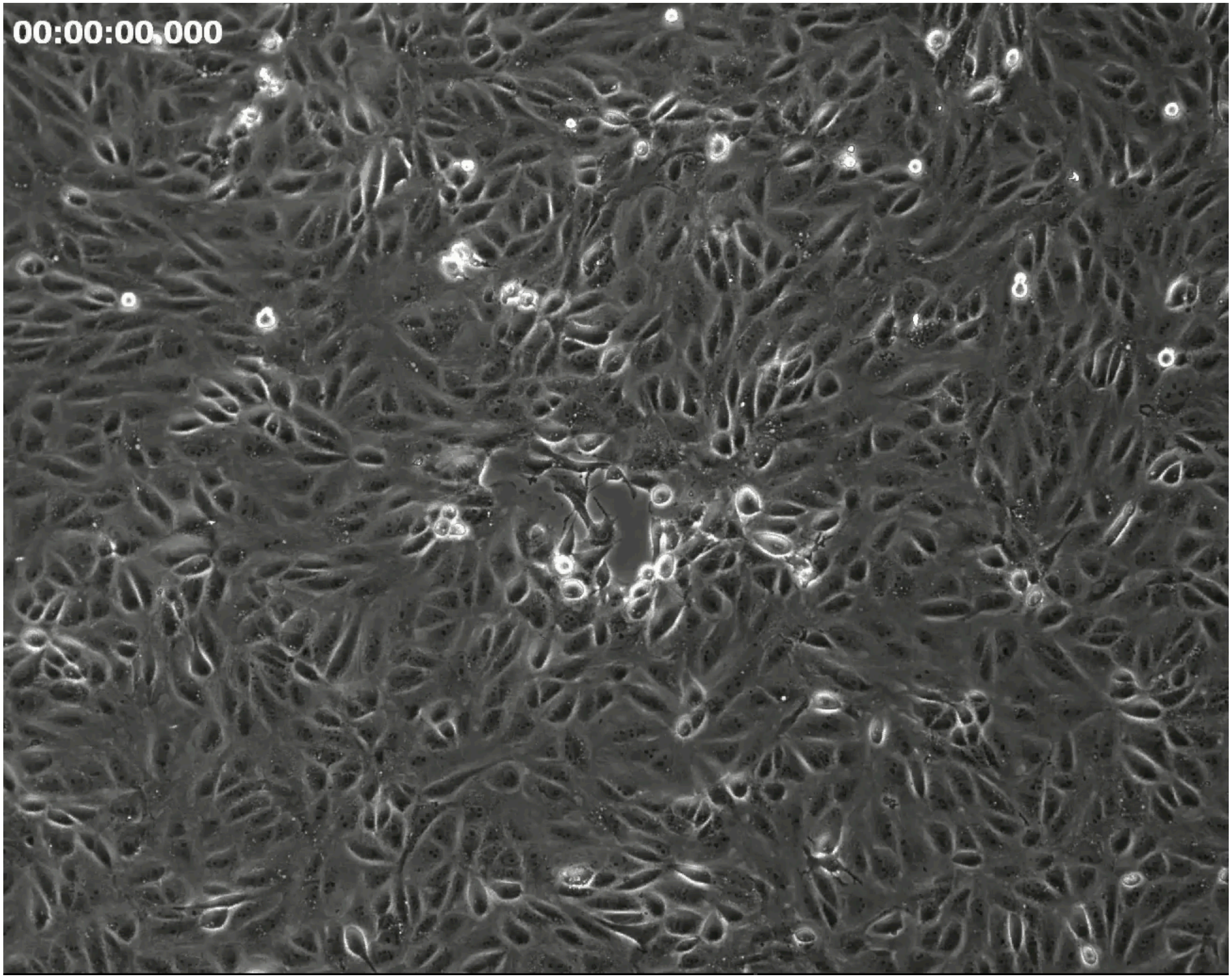
Plaque assay







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virology.ws/2013/06/15/the-wall-of-polio/

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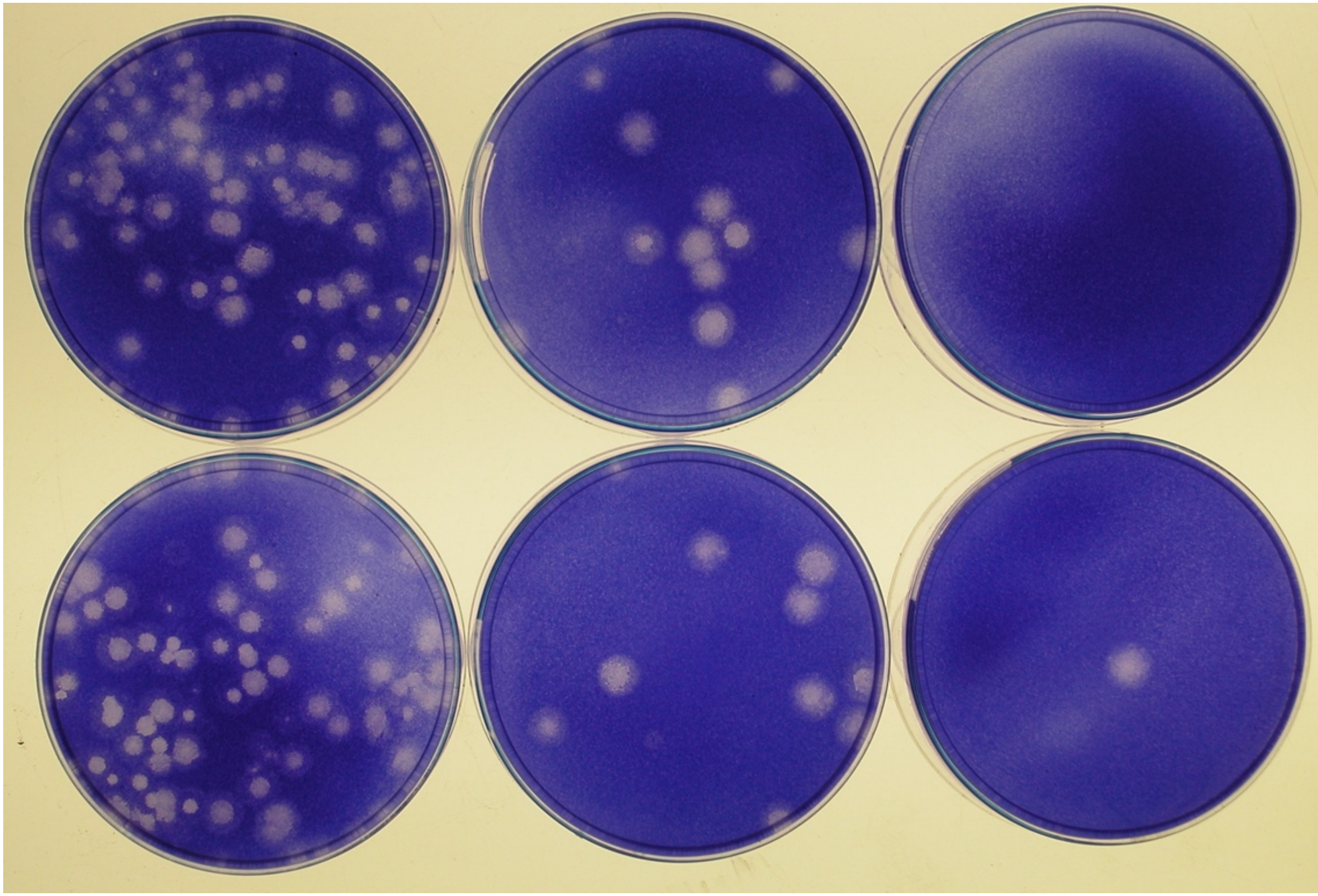
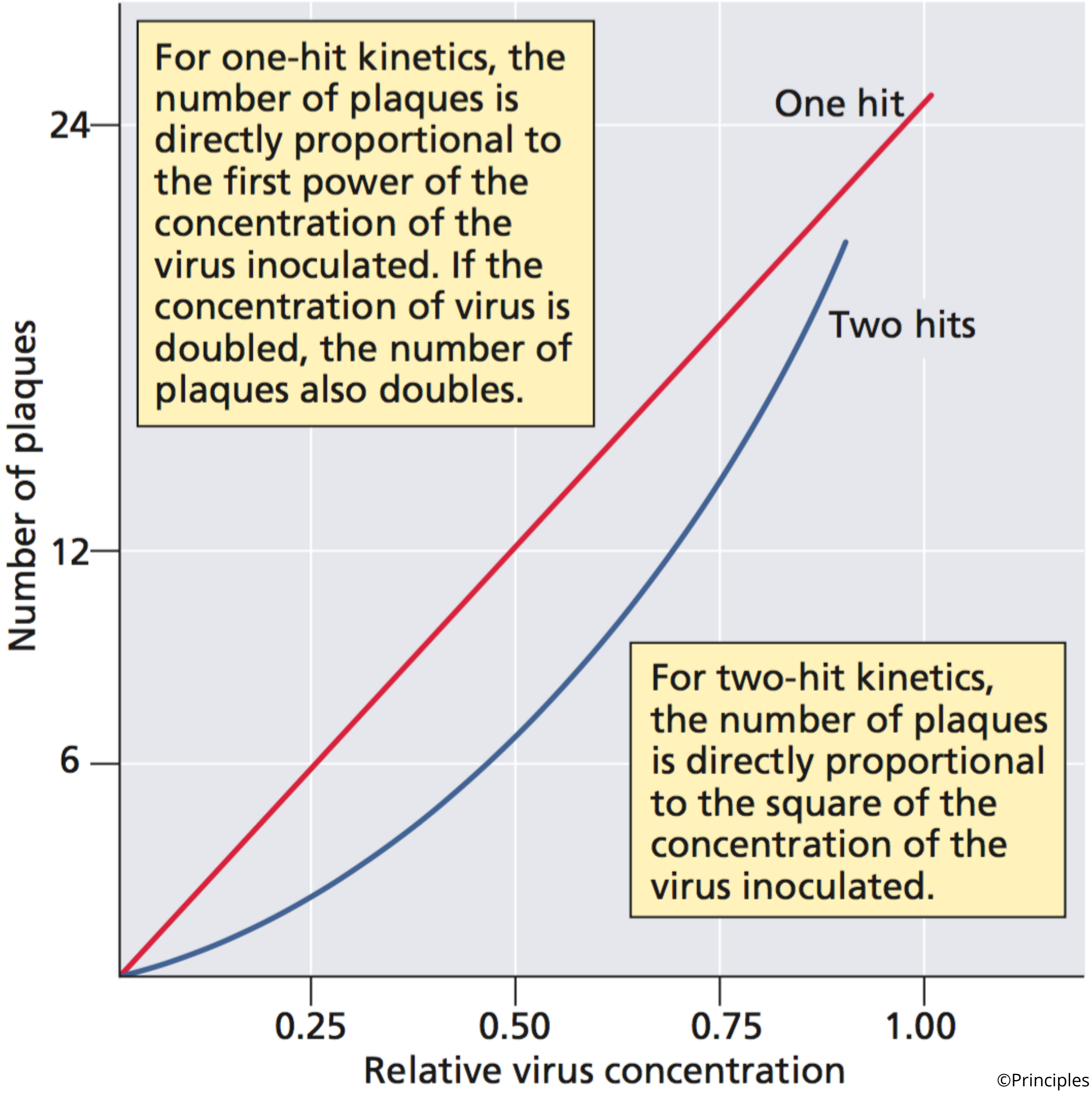
When doing a plaque assay, what is the purpose of adding a semi-solid agar overlay on the monolayer of infected cells?

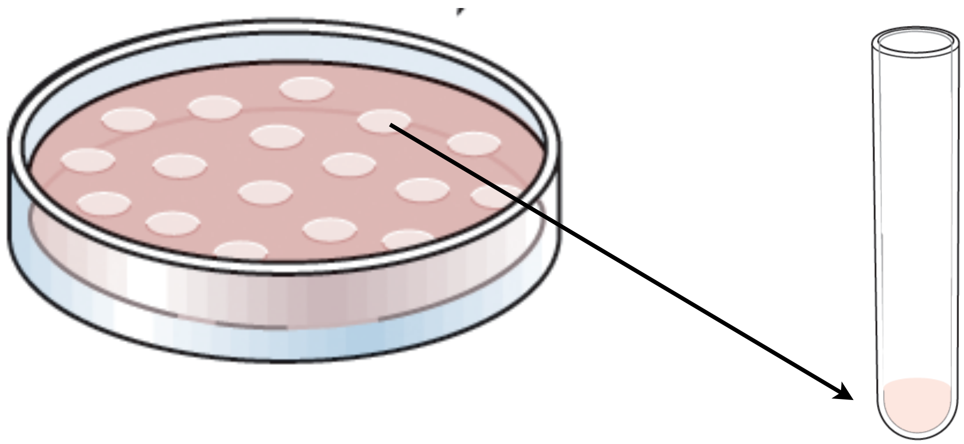
1. To stabilize progeny virions
2. To ensure that cells remain susceptible and permissive
3. To act as a pH indicator
4. To keep cells adherent to the plate during incubation
5. To restrict viral diffusion after lysis of infected cells



How many viruses are needed to form a plaque?

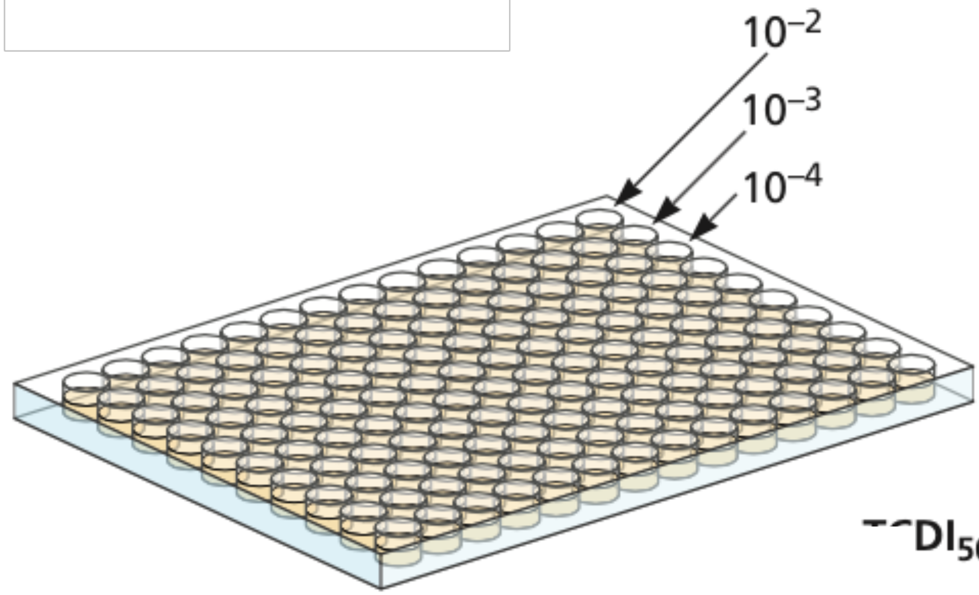

For one-hit kinetics, the number of plaques is directly proportional to the first power of the concentration of the virus inoculated. If the concentration of virus is doubled, the number of plaques also doubles.
One hit
Two hits
For two-hit kinetics, the number of plaques is directly proportional to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

Plaque purification



A method for producing clonal virus stocks
Usually done 3 times

Endpoint dilution assay



TCID$_{50}$

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

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

Particle-to-PFU ratios of 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

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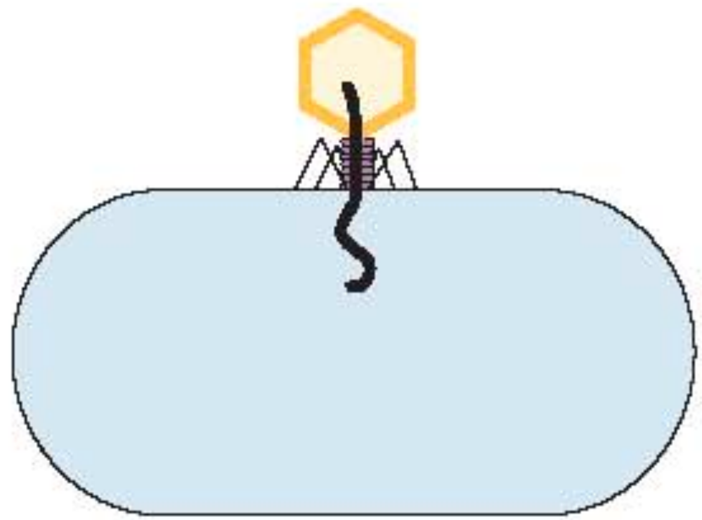
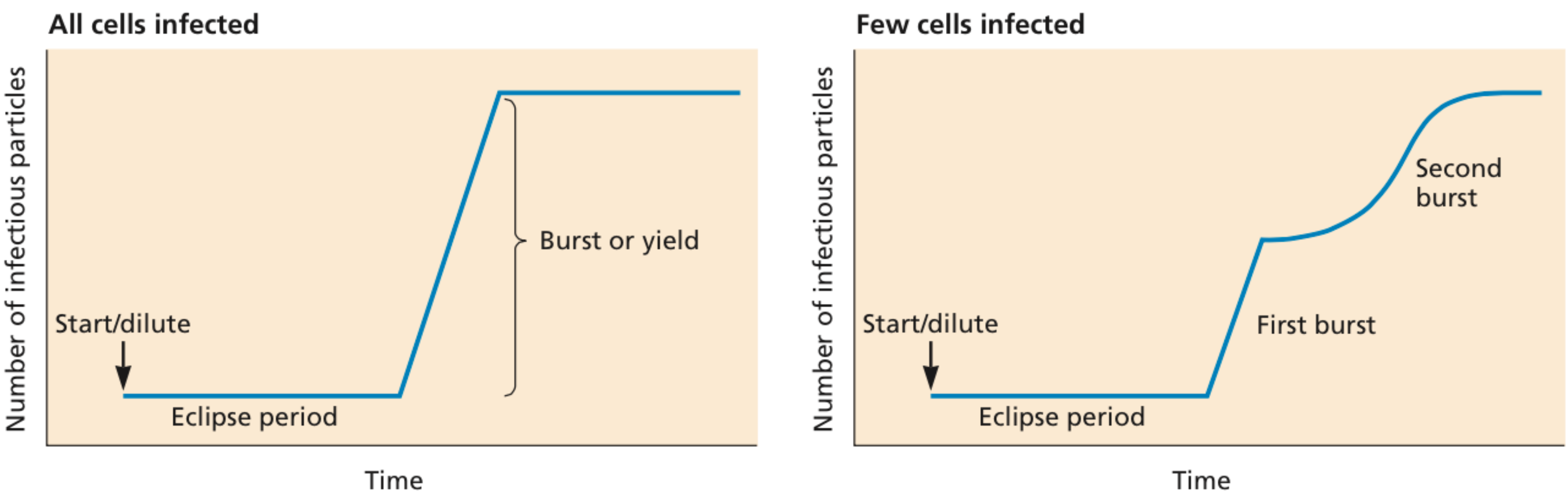
In the ‘particle to pfu ratio’, ‘particle’ can best be described as:

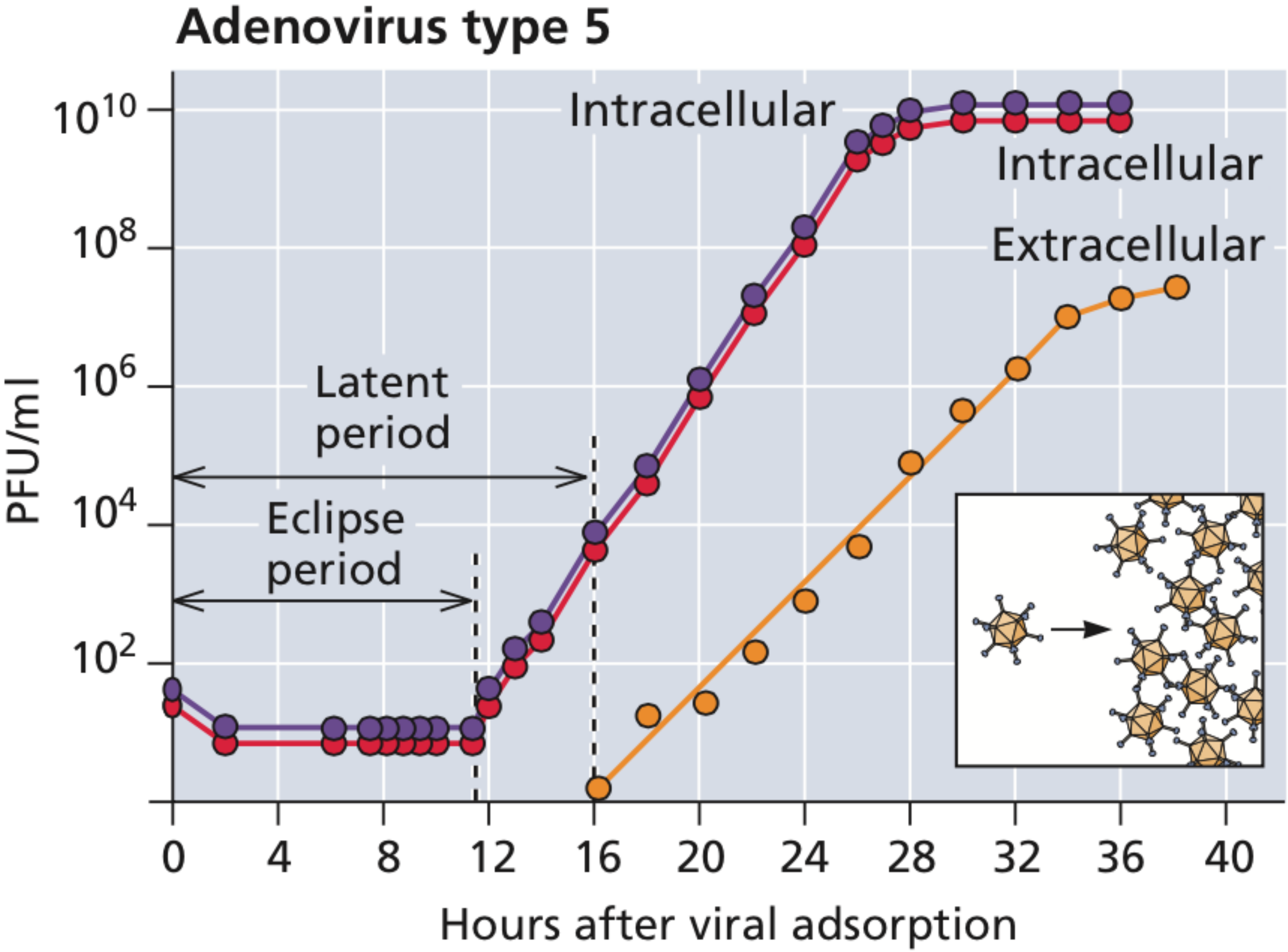
1. One of the proteins which makes up the virion
2. A virus which may or may not be infectious
3. A virus which is infectious
4. A virus which is not infectious
5. Elementary or composite

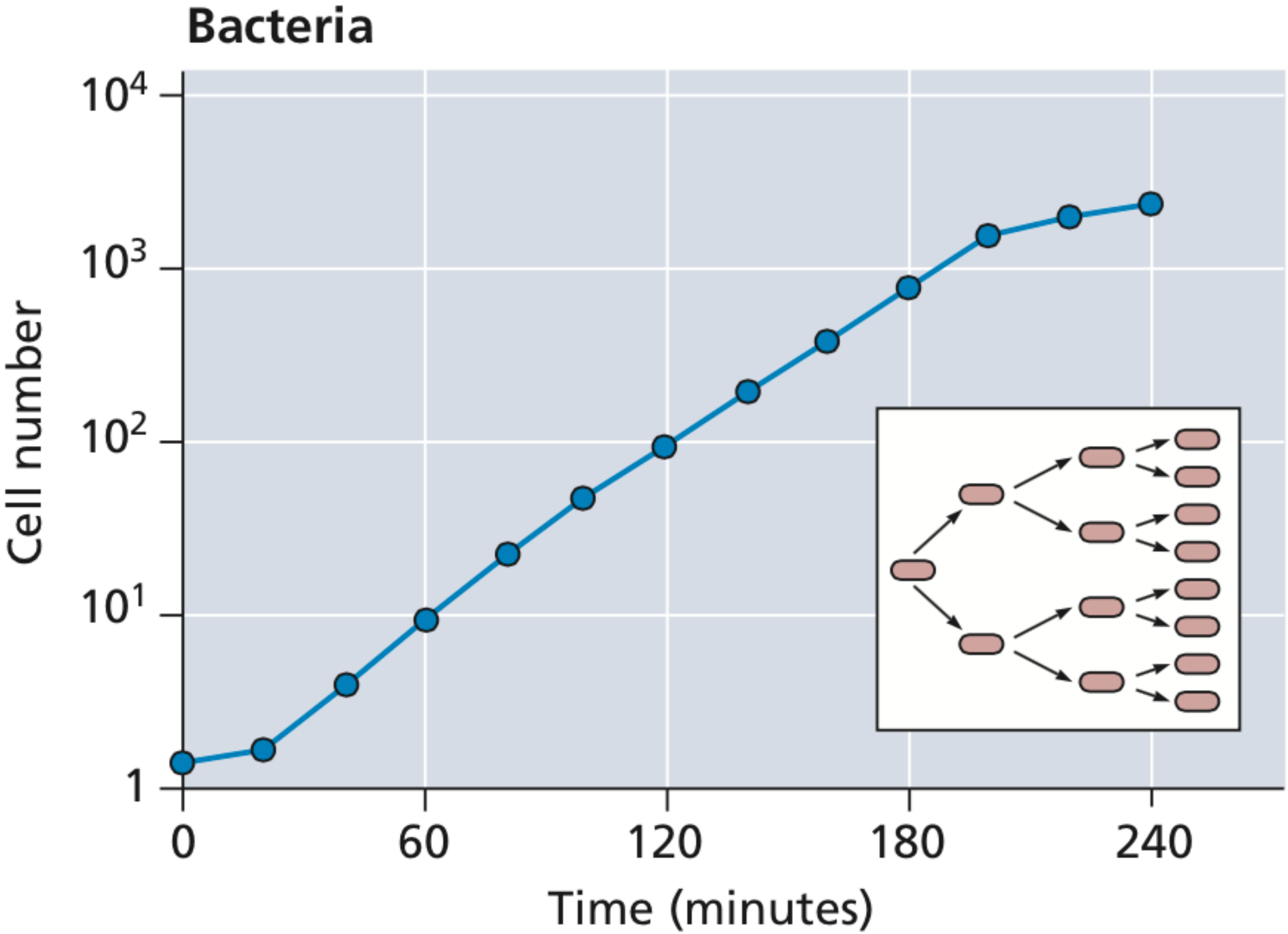
One-step growth cycle

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




All cells infected
Number of infectious particles
Start/dilute
Eclipse period
Burst or yield
Time
Few cells infected
Number of infectious particles
Start/dilute
Eclipse period
First burst
Second burst
Time

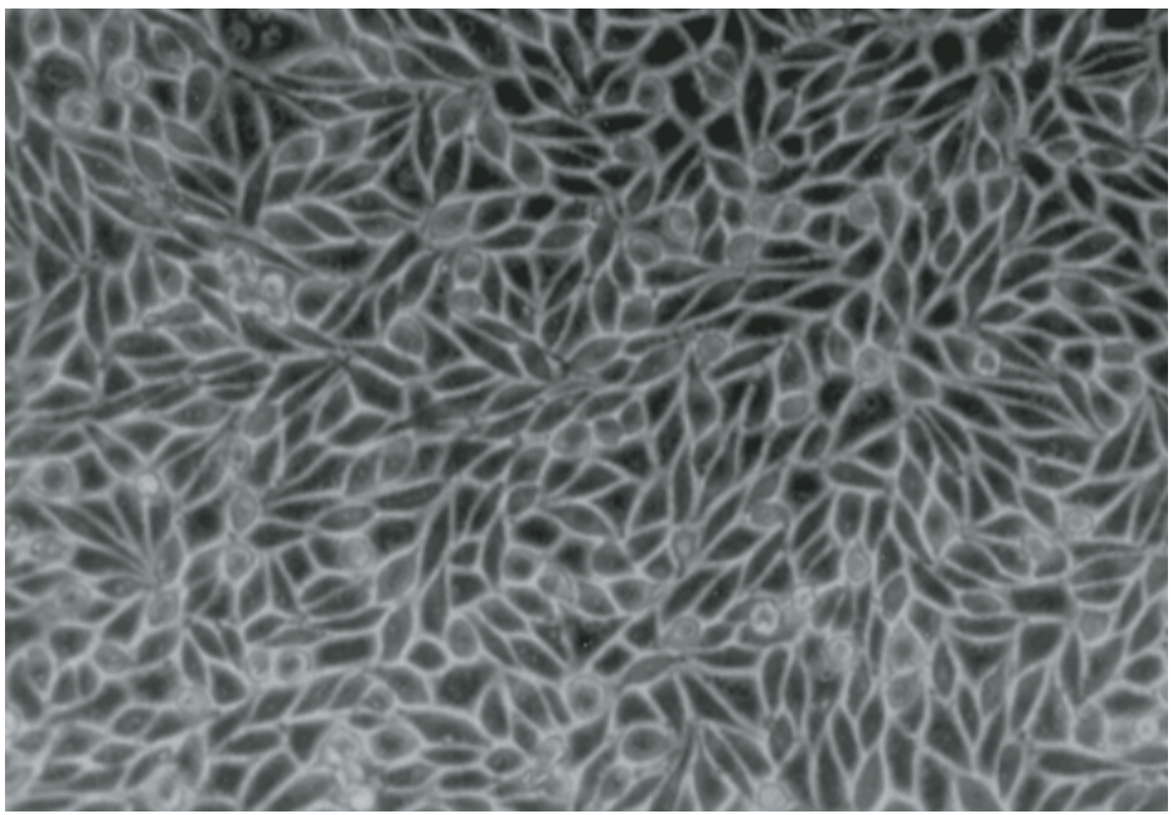

Adenovirus type 5
Intracellular
Intracellular
Extracellular
Latent
period
Eclipse
period
PFU/ml
10^{10}
10^{8}
10^{6}
10^{4}
10^{2}
0
4
8
12
16
20
24
28
32
36
40
Hours after viral adsorption



Bacteria
Cell number
10^4
10^3
10^2
10^1
1
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
- 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 virions

MOI

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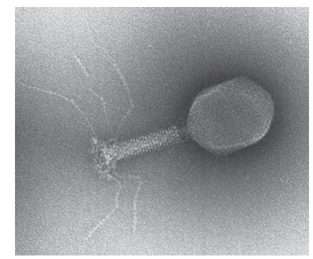
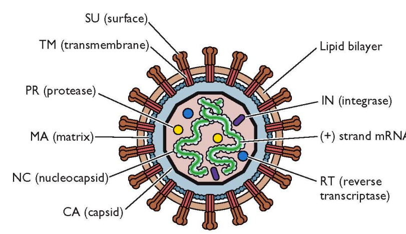
If cells are infected at an MOI=10 in a one-step growth cycle experiment, in the growth curve you will likely see…

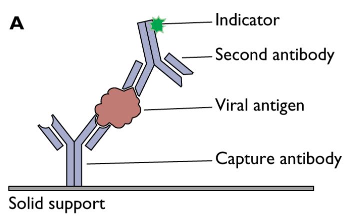
1. Multiple bursts of virus release
2. Multiple eclipse periods
3. A single burst of virus release
4. No burst of virus release
5. Asynchronous infection

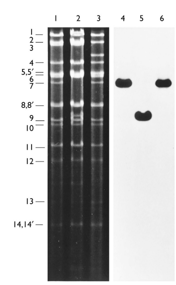
Physical measurements of virus

- Hemagglutination
- Electron microscopy
- Viral enzymes
- Serology
- Nucleic acids

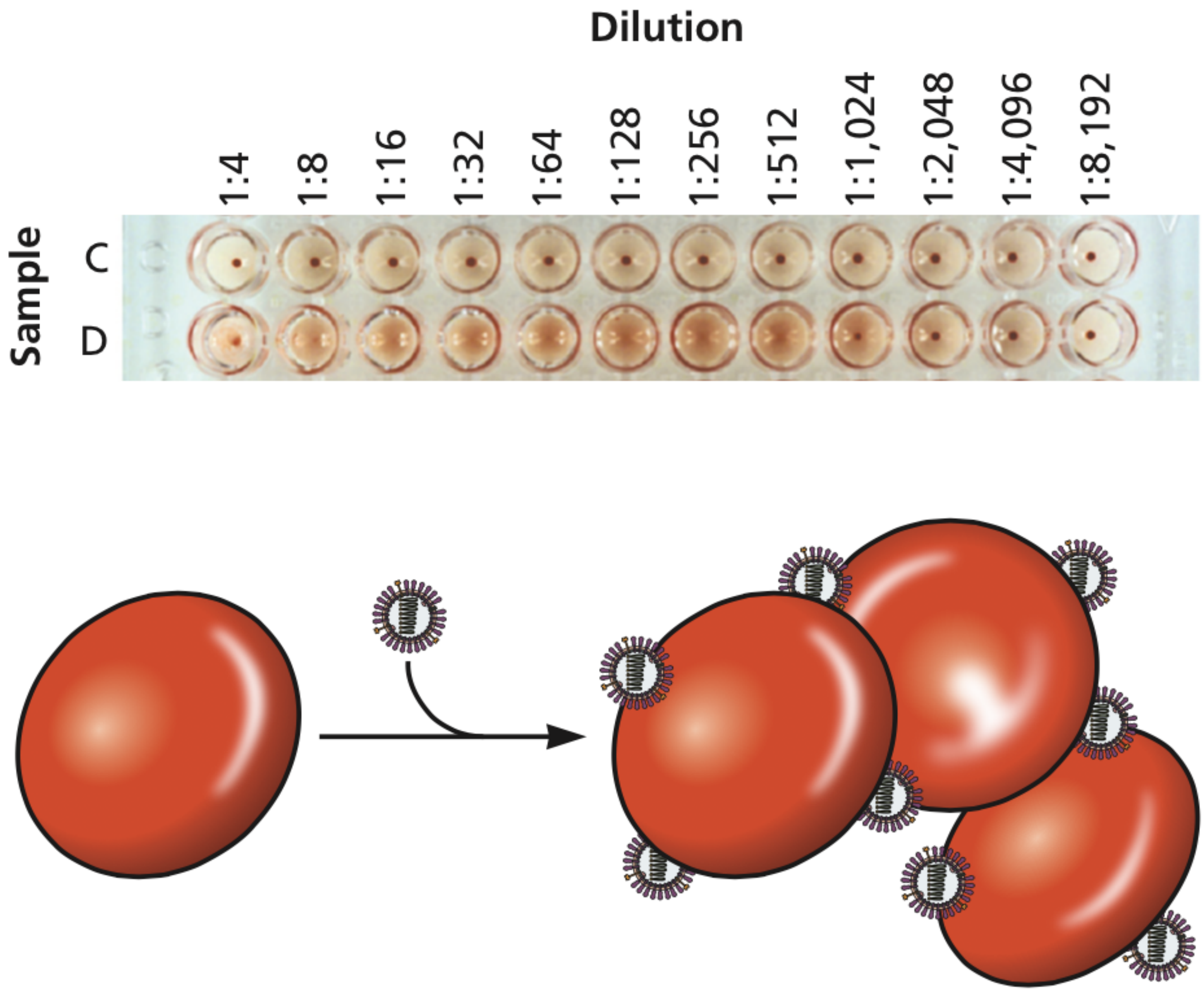




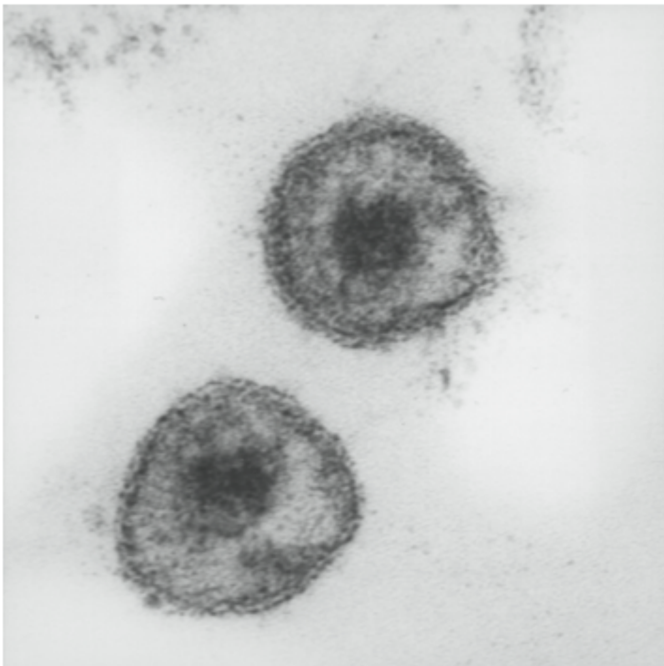


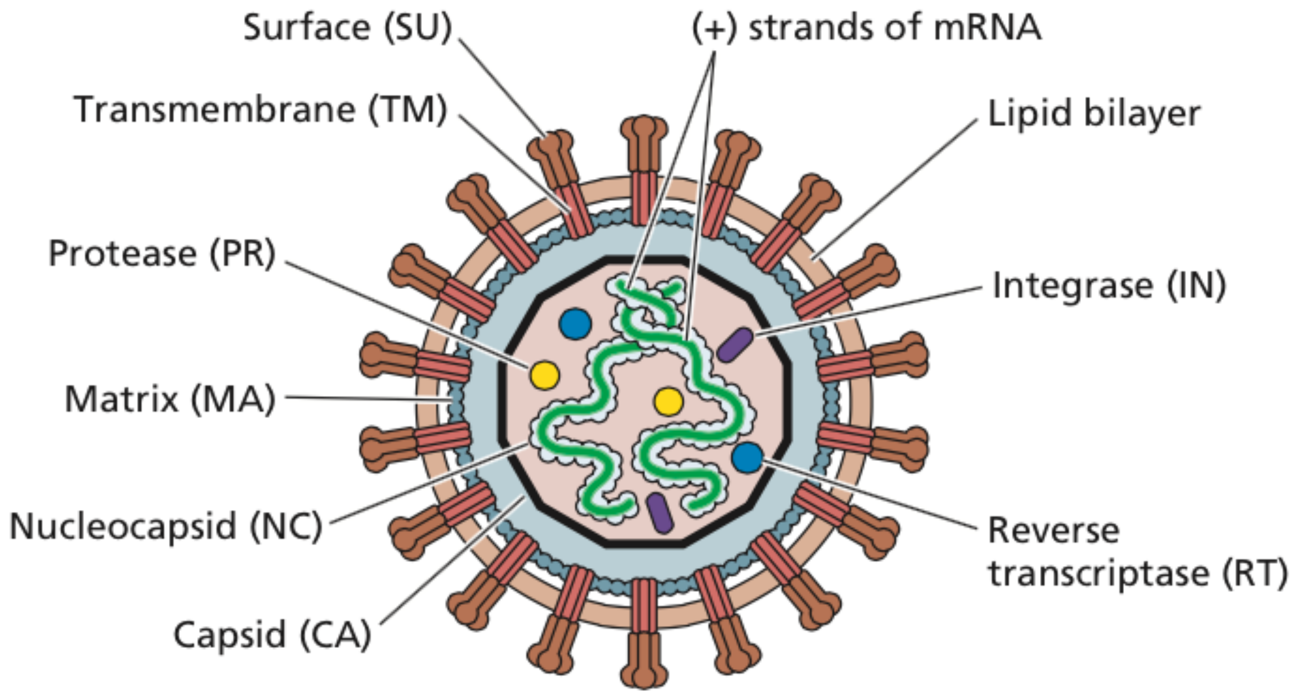


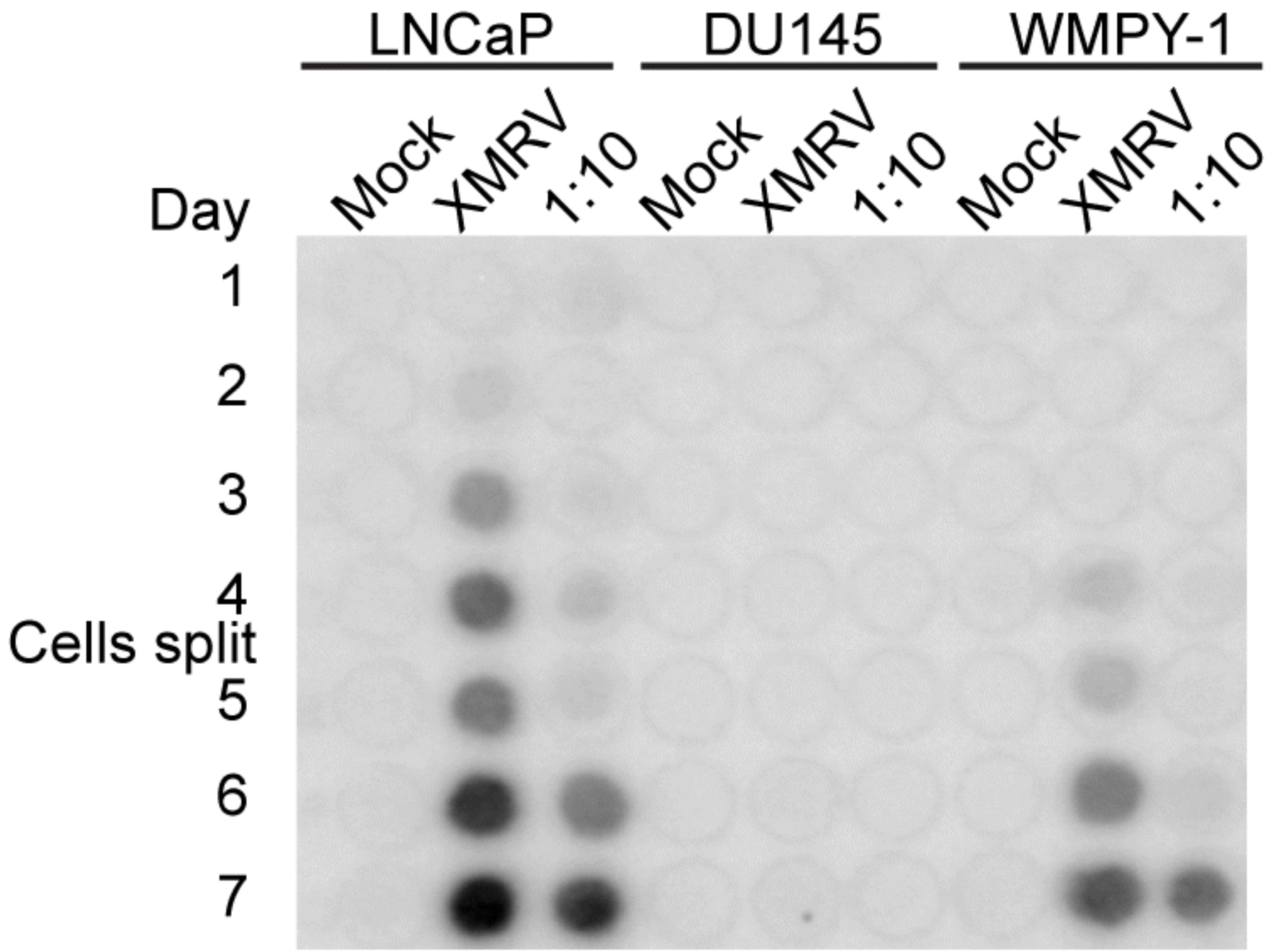
Hemagglutination



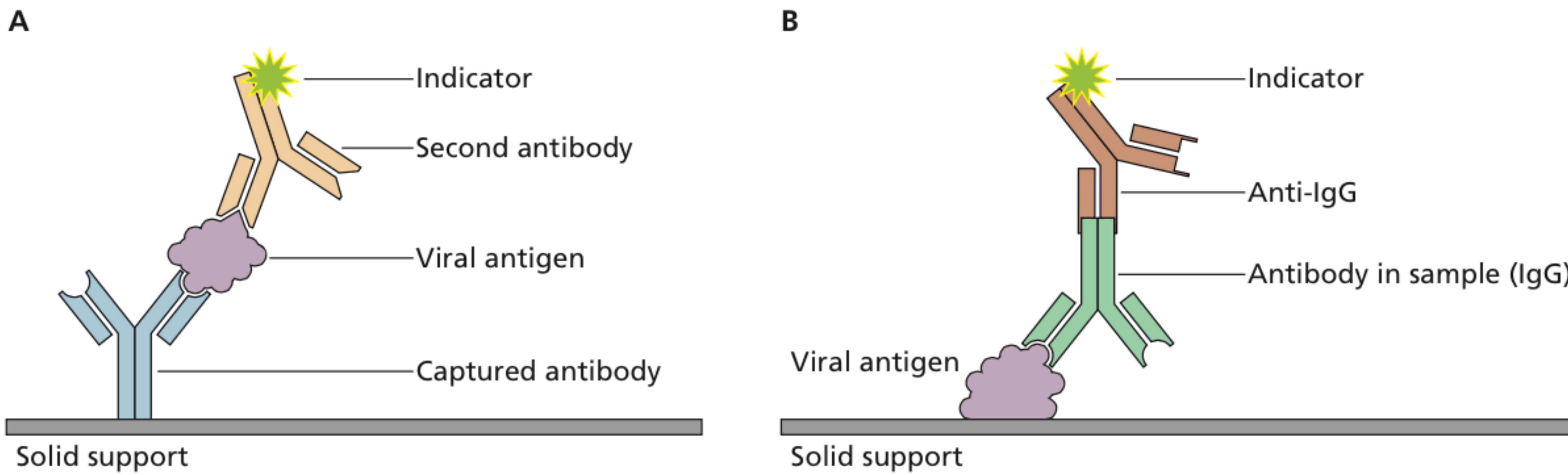
Measurement of viral enzyme activity

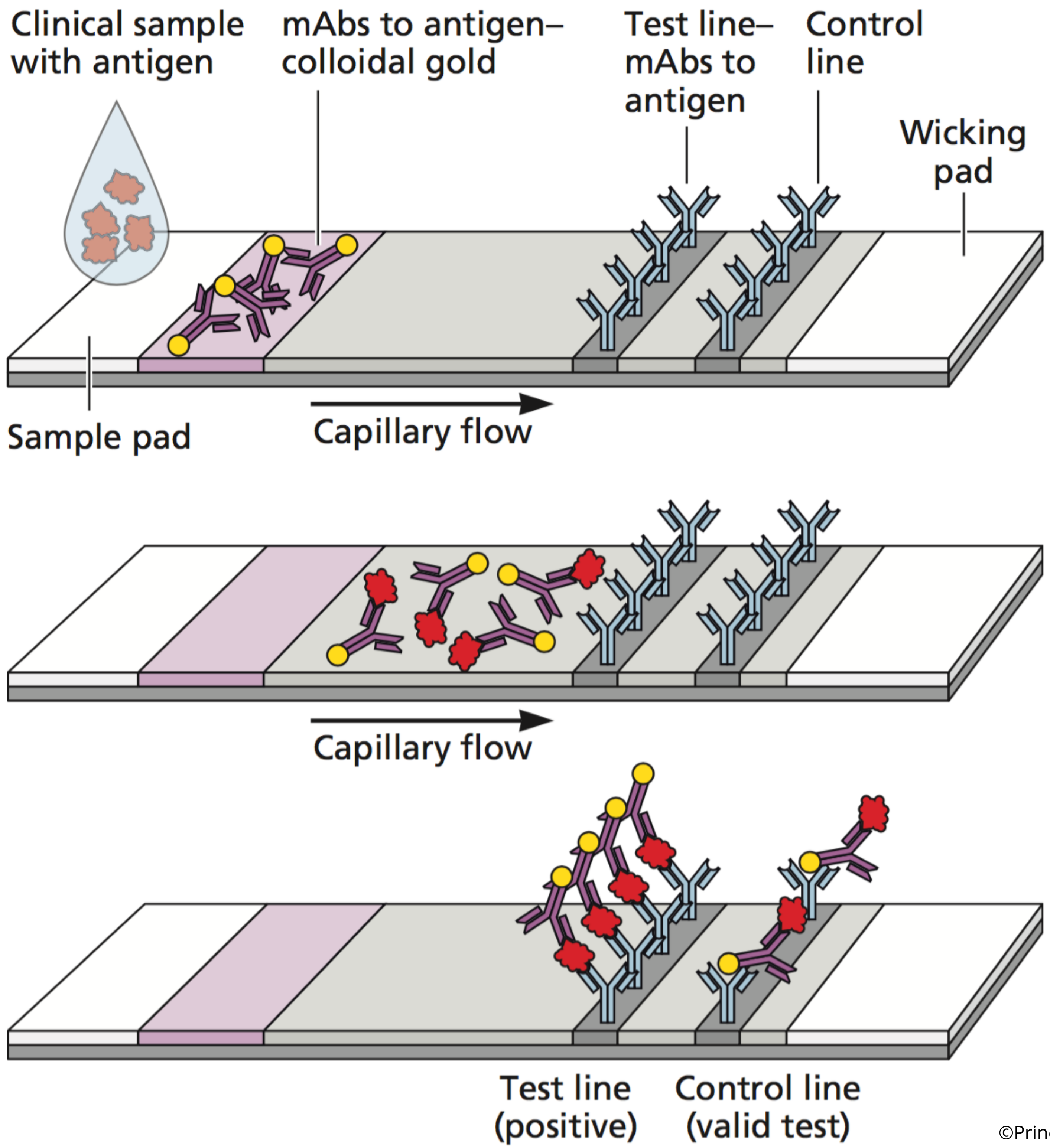


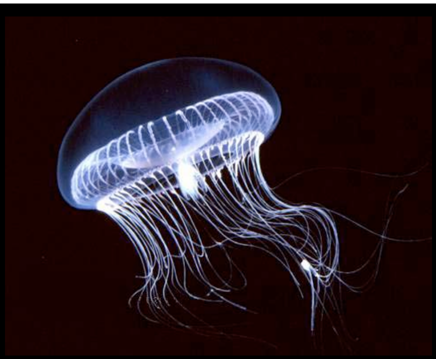



LNCaP
DU145
WMPY-1
Mock
XMRV
1:10
Mock
XMRV
1:10
Mock
XMRV
1:10
Day
1
2
3
4
Cells split
5
6
7

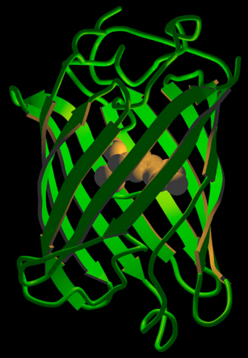
Enzyme-linked immunosorbent assay (ELISA): detecting viral antigens or antibodies

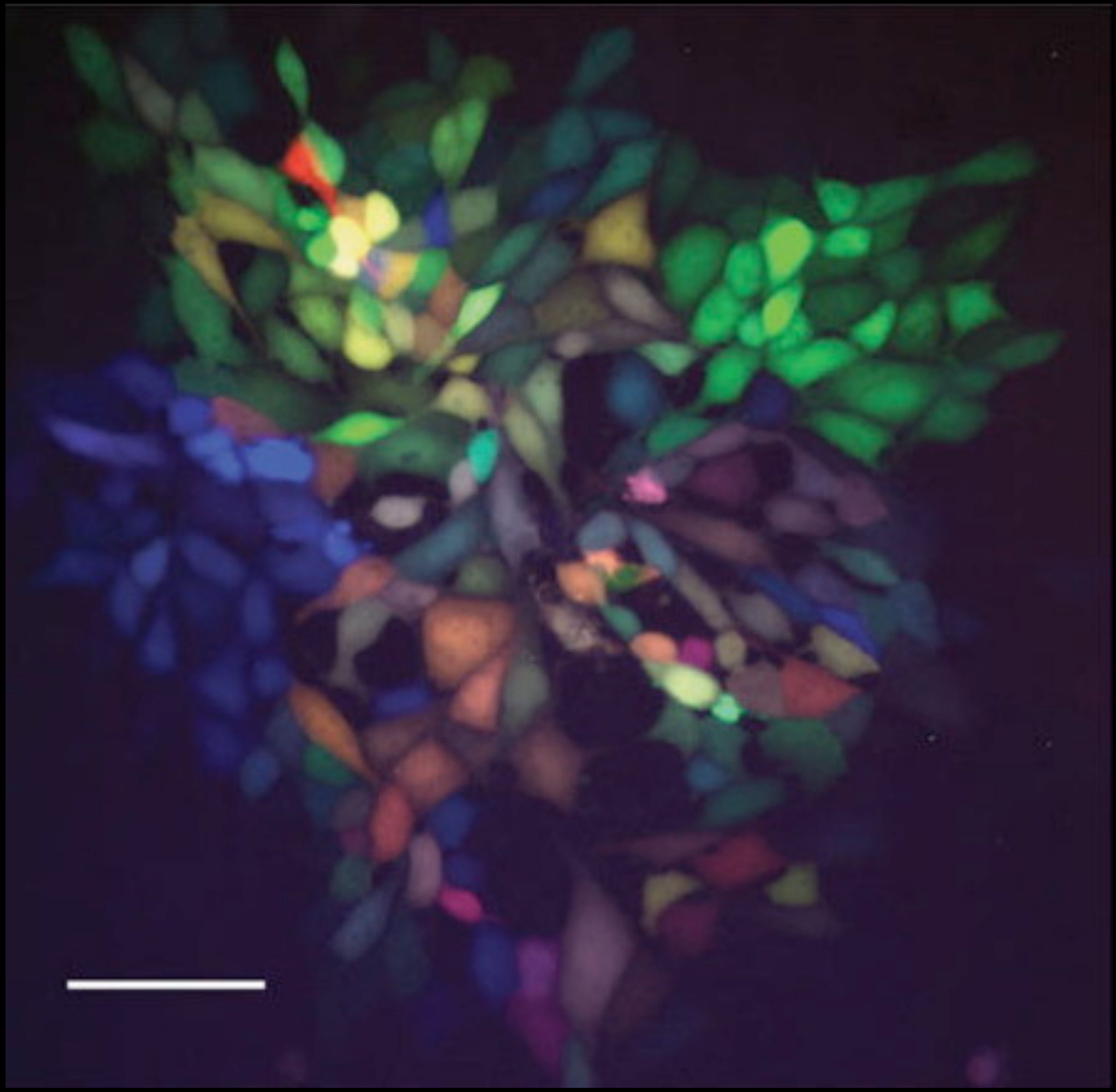


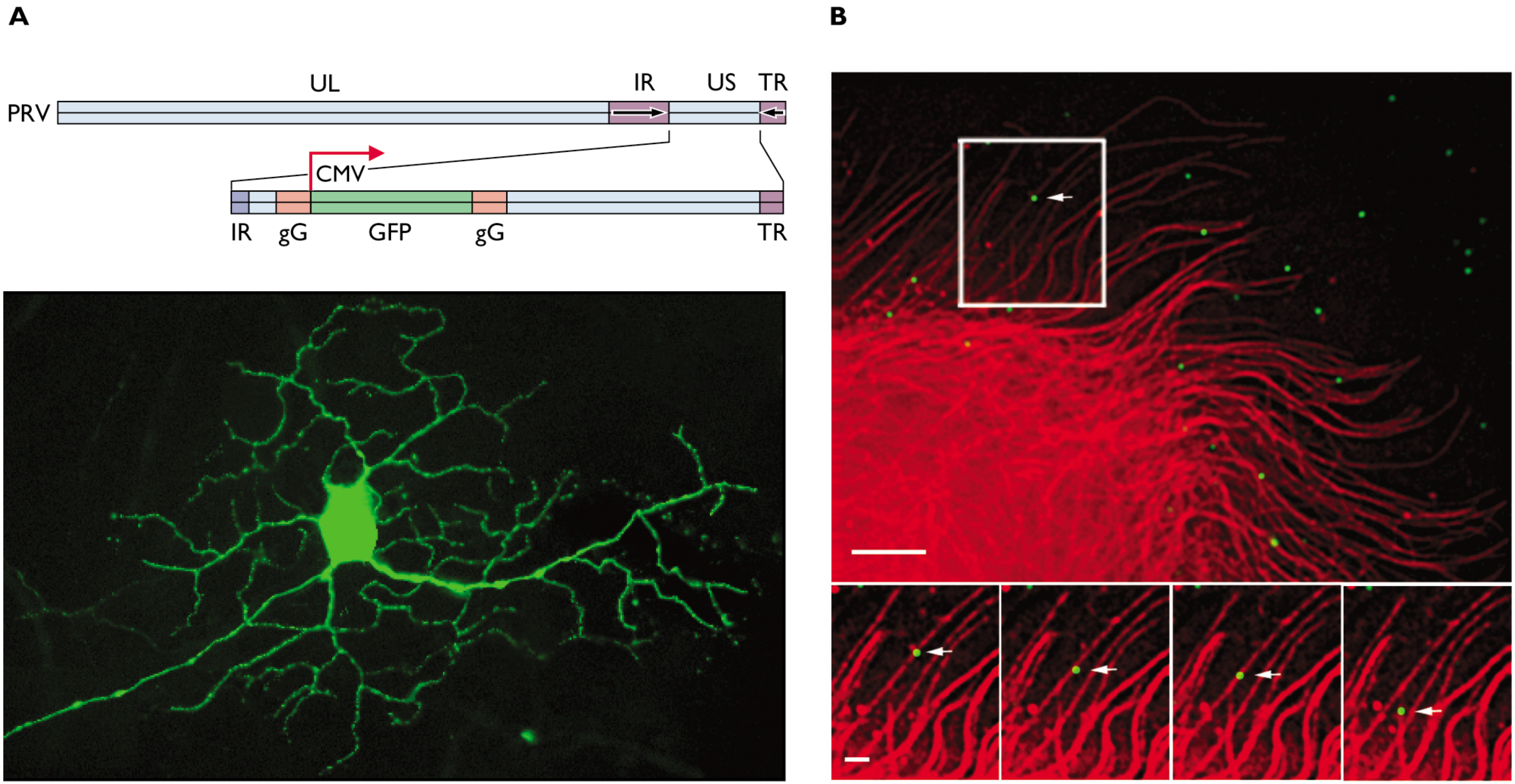

Clinical sample with antigen
mAbs to antigen–colloidal gold
Test line–mAbs to antigen
Control line
Wicking pad
Sample pad
Capillary flow
Capillary flow
Test line (positive)
Control line (valid test)



Green fluorescent protein

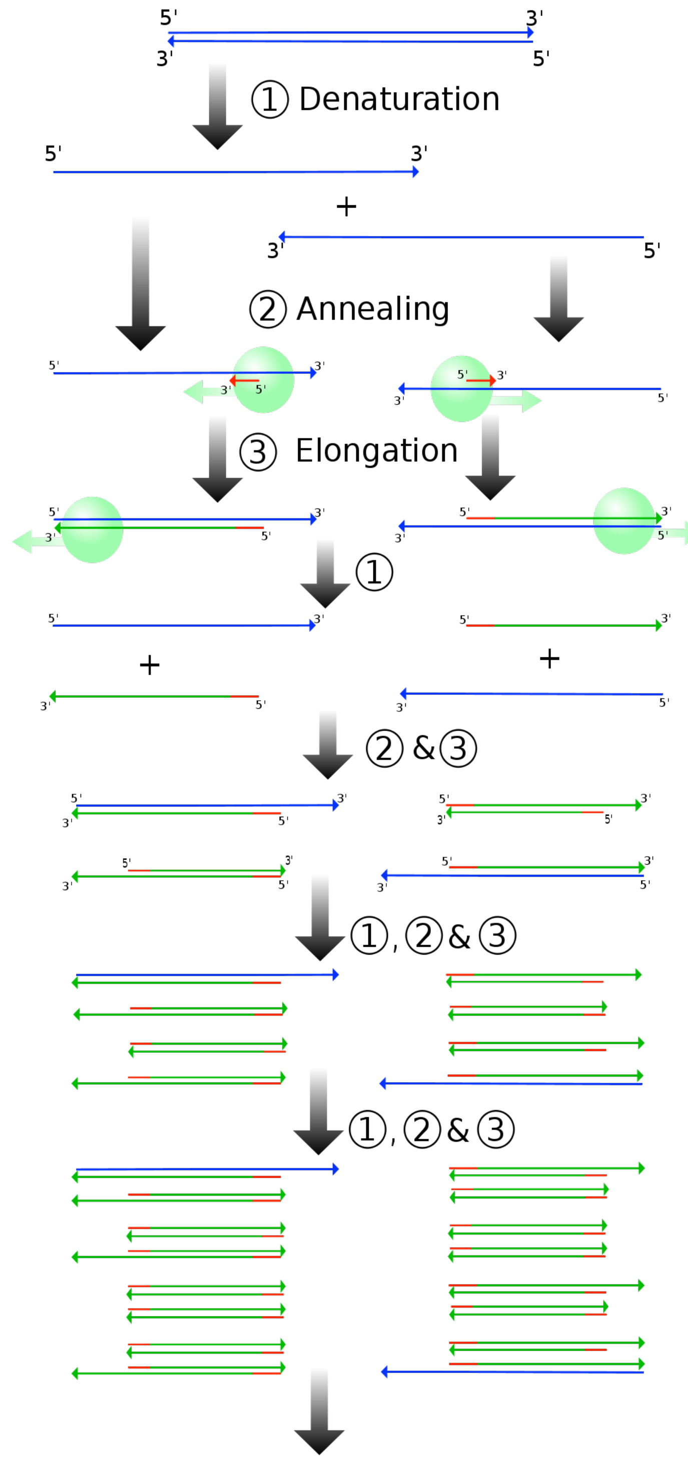





A
B
UL
IR
US
TR
PRV
CMV
IR
gG
GFP
gG
TR

Polymerase chain reaction (PCR)

- Research
- Industry
- Diagnosis



Deep, high-throughput sequencing

- Metagenomics
- Identification of new viruses in environmental samples
- Identification of new pathogens
- Human genome: 10 yr/$3B vs 1 day/$1500

(this is not DHTS)

TWiV 196: An arena for snakes

AUGUST 19, 2012

Hosts: Vincent Racaniello, Alan Dove, Rich Condit, Dickson Despommier, Kathy Spindler, Mark Stenglein, and Joseph DeRisi

The TWiVites meet with Mark Stenglein and Joseph DeRisi to discuss their discovery of a novel arenavirus in snakes with inclusion body disease.

http://www.twiv.tv/2012/08/19/twiv-196-an-arena-for-snakes/

TWiV 199: Of mice, ticks, and pigs

SEPTEMBER 16, 2012

Hosts: Vincent Racaniello, Alan Dove, Rich Condit, and Kathy Spindler

Vincent, Alan, Rich, and Kathy discuss recent outbreaks of hantavirus pulmonary syndrome in Yosemite National Park and novel swine-origin influenza in the US midwest, and isolation of the Heartland virus from two patients in Missouri with severe febrile illness.

TWiV 0:00

Click the arrow above to play, or right-click

http://www.twiv.tv/2012/09/16/twiv-199-of-mice-ticks-and-pigs/

Viruses and viral sequences

RESEARCH ARTICLE OPEN ACCESS

Zoonotic Viruses Associated with Illegally Imported Wildlife Products

January 13, 2012, 8:10 AM

From the Jungle to J.F.K., Viruses Cross Borders in Monkey Meat

By RACHEL NUWER

Carlos Rodriguez/Wildlife Conservation Society

A set of Cercopithecus monkey limbs recently confiscated at O'Hare Airport in Chicago.

Science

It's a familiar story: deep within the jungle, an intrepid explorer or hunter awakens something he shouldn't have. Maybe he bagged the wrong bat or came into contact with an ailing chimp. Whatever the origin, he unsuspectingly becomes host to something deadly and unseen and unwittingly carries it back home across the river or the ocean.

And then the pandemic begins.

http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0029505