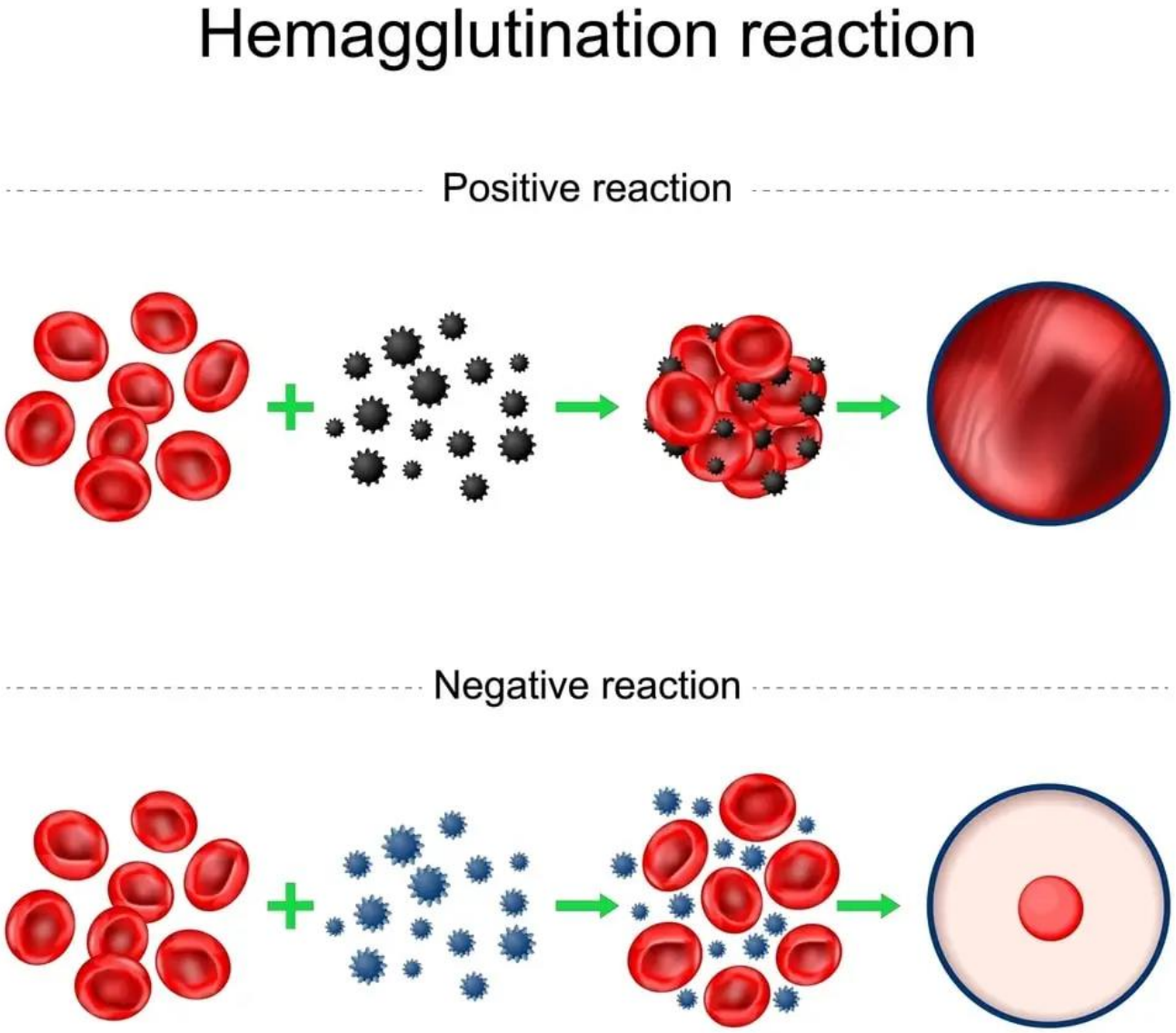
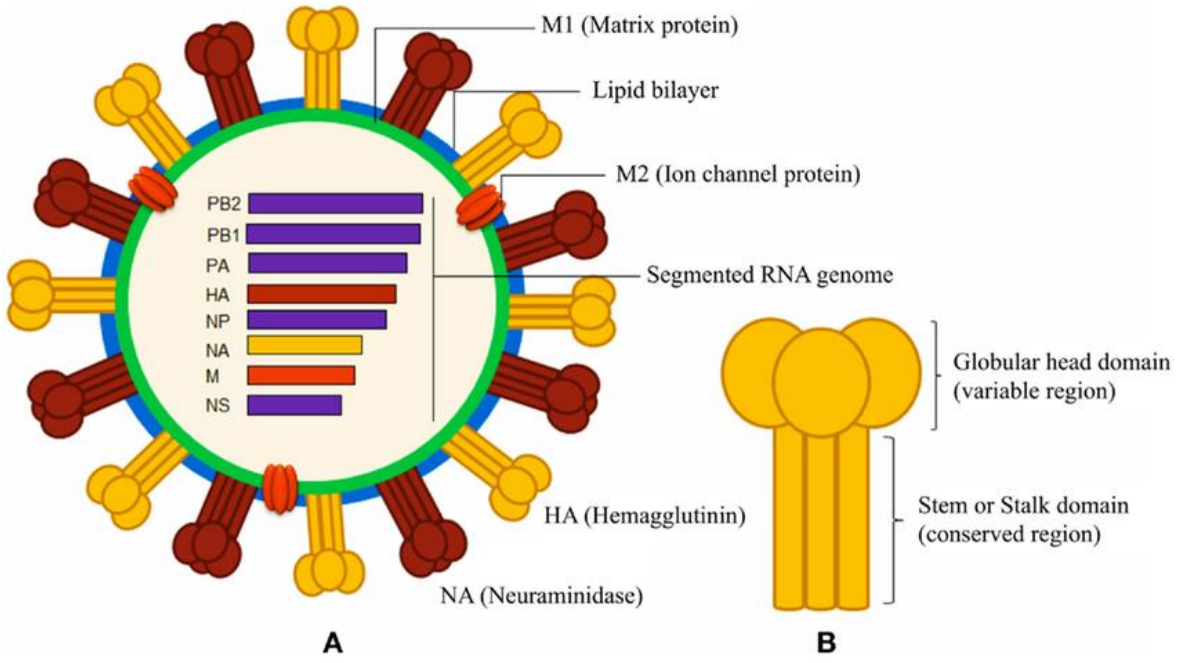
Hemagglutination (HA)

- The agglutination of red blood cells by **viruses**



HA test (Takahara et al. 2019) https://microbialnotes.com/hemagglutination-assay-principle-interpretation

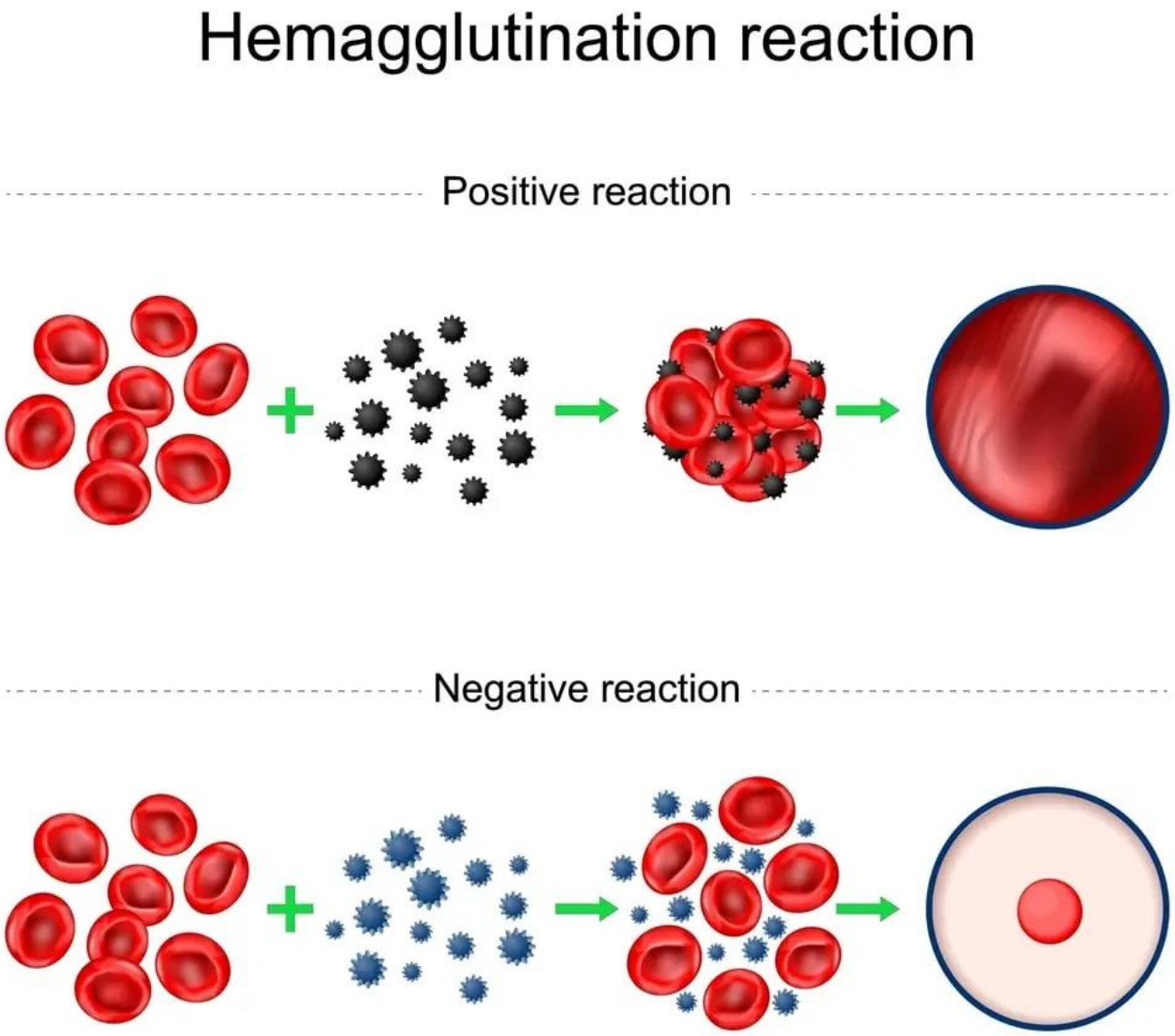
- The principle behind the hemagglutination test is that the nucleic acids of viruses encode proteins, such as hemagglutinin, that are expressed on the surface of the virus.



- As the name implies, these hemagglutinin proteins expressed on the surface of the virus bind to or clump erythrocytes creating a lattice, which settle irregularly in the bottom of the test tube or the microtiter well.

Lofano, Giuseppe & Kumar, Arun & Finco, Oretta & Del Giudice, Giuseppe & Bertholet, Sylvie. (2015). B Cells and Functional Antibody Responses to Combat Influenza. Frontiers in immunology. 6. 336. 10.3389/fimmu.2015.00336.

- Hemagglutinin, interacts with red blood cells, causing them to clump and form a lattice.
- Red blood cells precipitate to form a red dot at the bottom of a container in the absence of an enveloped virus.
- However, in the presence of a virus, red blood cell clusters are dispersed and do not form a red dot.
- This is the basic concept of hemagglutination tests.



HA test (Takahara et al. 2019) https://microbialnotes.com/hemagglutination-assay-principle-interpretation

- Mostly enveloped viruses (such as orthomyxo, pox, influenza, paramyxo) have this feature.

- Also some non-envelope viruses (such as parvovirus, adenovirus) have hemagglutination properties.

HA-red blood cell relationship

- The hemagglutination assay and hemagglutination inhibition assay use the hemagglutination process, which involves the adhesion of sialic acid receptors on the surface of red blood cells (RBCs) with the hemagglutinin glycoprotein present on the surface of certain viruses, including the influenza virus and others.
- The ability of the virus to hemagglutinate is limited to the species from which erythrocytes are obtained.

For example,

- Adenovirus + human 0 group and rat erythrocyte
- Newcastle V + Chicken erythrocyte

→ HA

https://microbialnotes.com/hemagglutination-assay-principle-interpretation

Preparation of erythrocyte suspension

- Anticoagulated blood centrifuged for 5-10 minutes at 2000 rpm.
- After centrifugation, the plasma and leukocyte layer is discarded, and the erythrocytes remaining in the bottom of the tube are washed 3 times with 0.85% PBS solution.
- After the last wash, the erythrocytes inside the tube are accepted as 100% and diluted to 0.5-1% (with PBS or saline) according to the purpose.
- The erythrocyte suspension can be stored at + 4 °C for up to 1 week.

Hemagglutination can be performed in two ways according to purpose

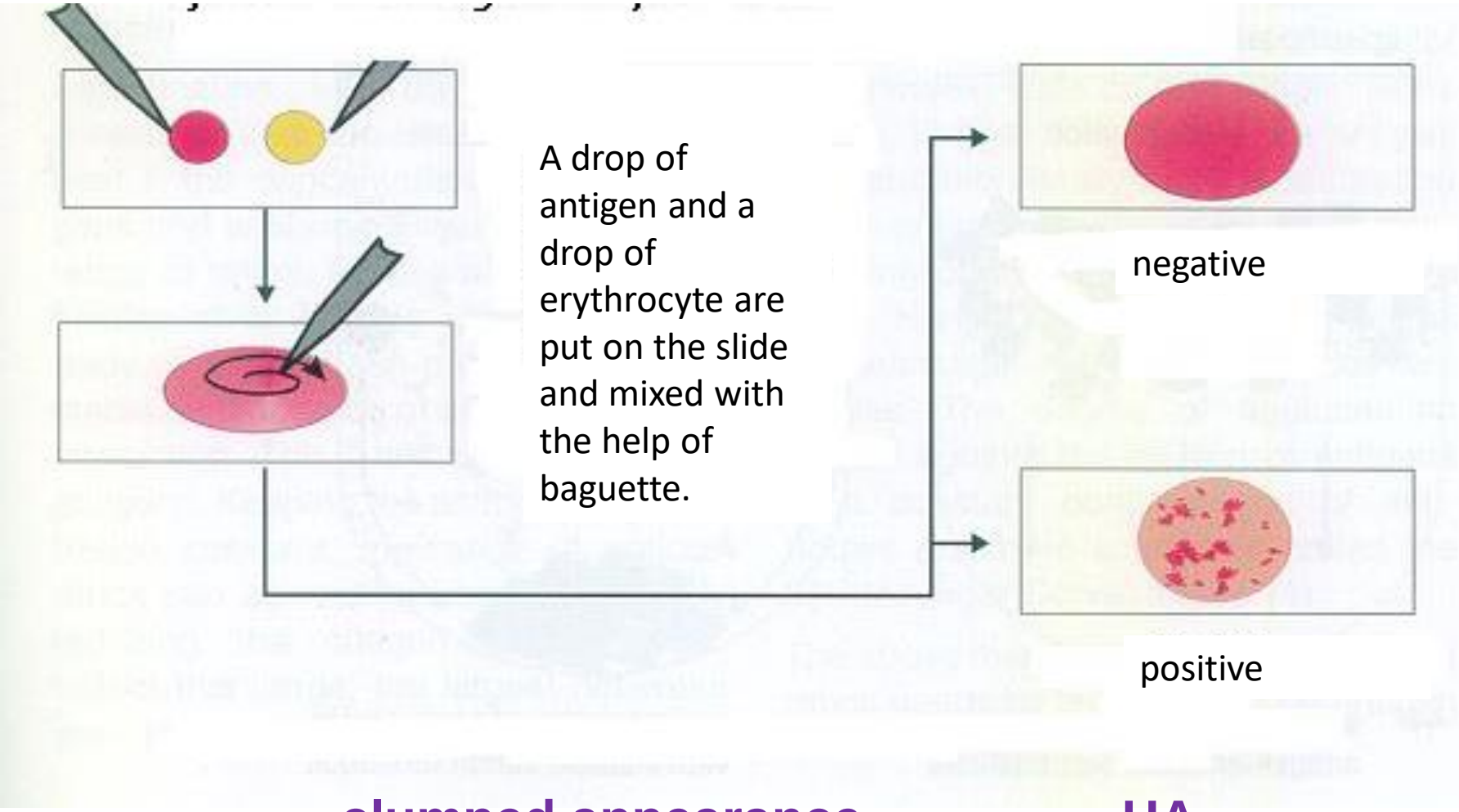
1. Rapid HA (on the slide):

- Qualitative evaluation → positive or negative
- It can be understood whether the virus has HA ability and which types of erythrocytes it has this ability against.
- HA titer can not be determined.

2. Slow HA (in tubes) :

- It is suitable for quantitative evaluation.
- The titer of the virus is determined.

1. Rapid HA (on the slide):



RESULTS

Agglutinated red blood cells have a clumped appearance distinct from non-agglutinated red blood cells.

clumped appearance
(+)
(-)

HA
(+)
(-)

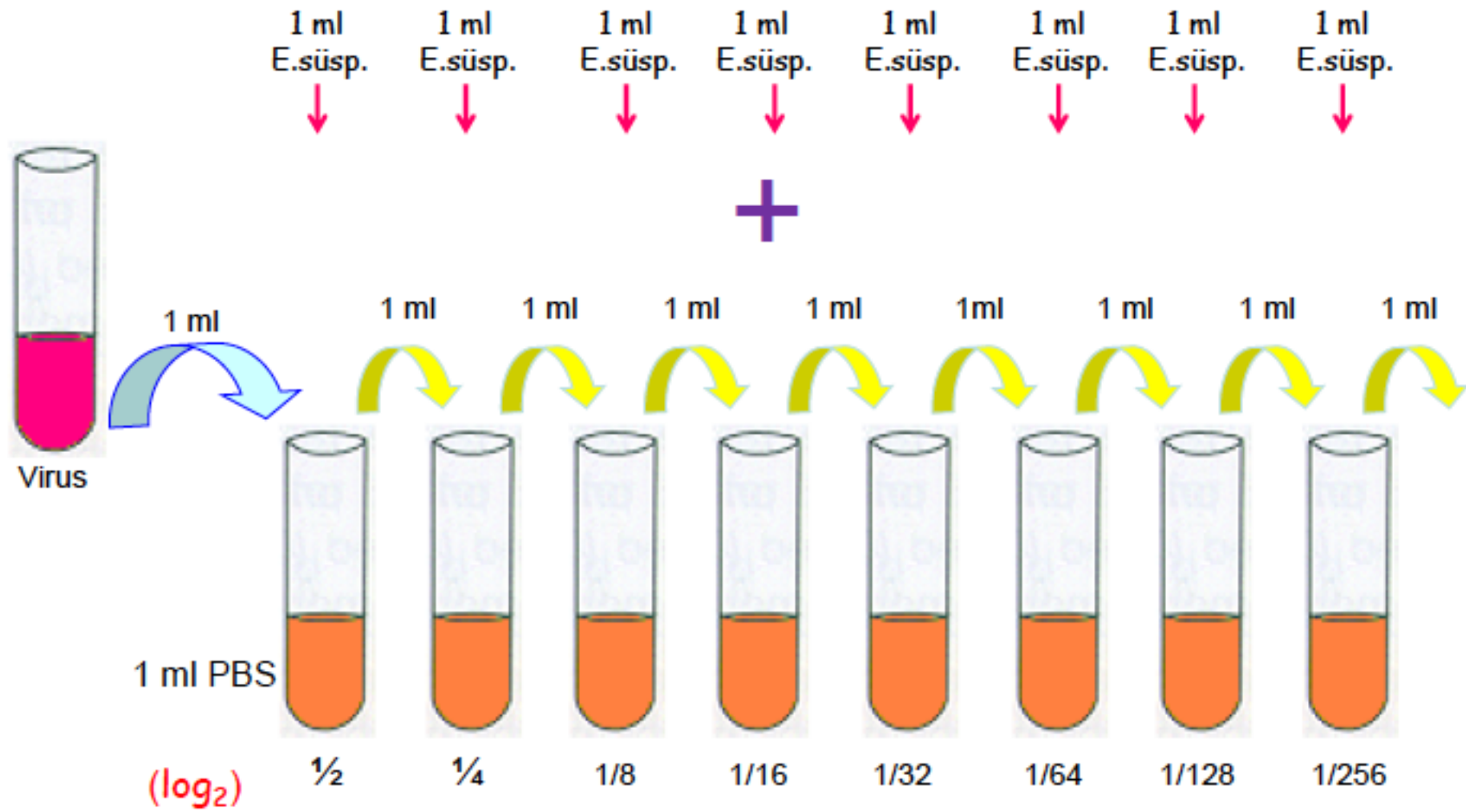
C.Staak, F.Salchow, N.Denzin : Practical Serology from the Basics to the testing, 2001.

2. Slow HA Assay

- HA feature of the virus can be detected,
- HA titer of the virus can be calculated,
- Identification of an isolated virus
- Standardization of virus to be used for HI test

Principle;

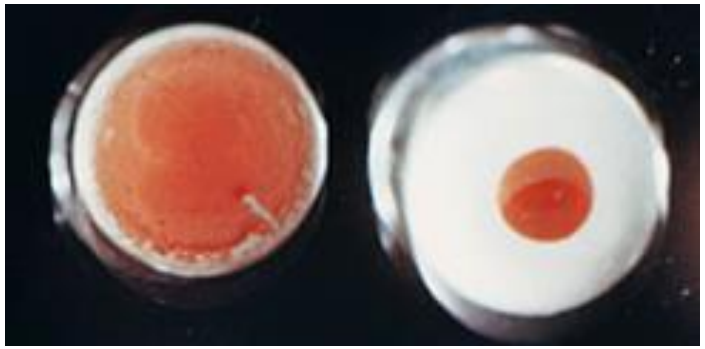
- A serial dilution of the virus is performed in the tube or **V-bottom microwell plate** (96 wells) according to the $Log_{2.}$
- 0.5 % erythrocyte suspension (equal amount to virus dilition) is added to all tubes or wells,
- 2 hours incubation period in room temperature.
- Finally, the result is evaluated based on the image at the bottom of the tubes or wells.


1 ml E.süsp.
1 ml E.süsp.
1 ml E.süsp.
1 ml E.süsp.
1 ml E.süsp.
1 ml E.süsp.
1 ml E.süsp.
1 ml E.süsp.
+
1 ml
Virus
1 ml
1 ml
1 ml
1 ml
1ml
1 ml
1 ml
1 ml
1 ml PBS
(log2)
½
¼
1/8
1/16
1/32
1/64
1/128
1/256

Evaluation of HA test;

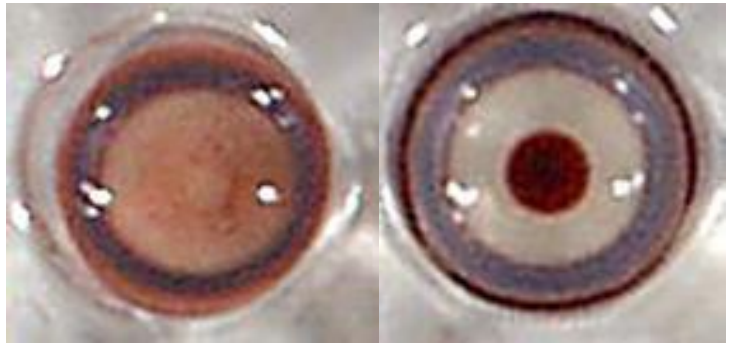
- GRID-DIFFUSE (reddish) style image HA (+)
- BUTTON (dot)-style display HA (-)

In tubes:

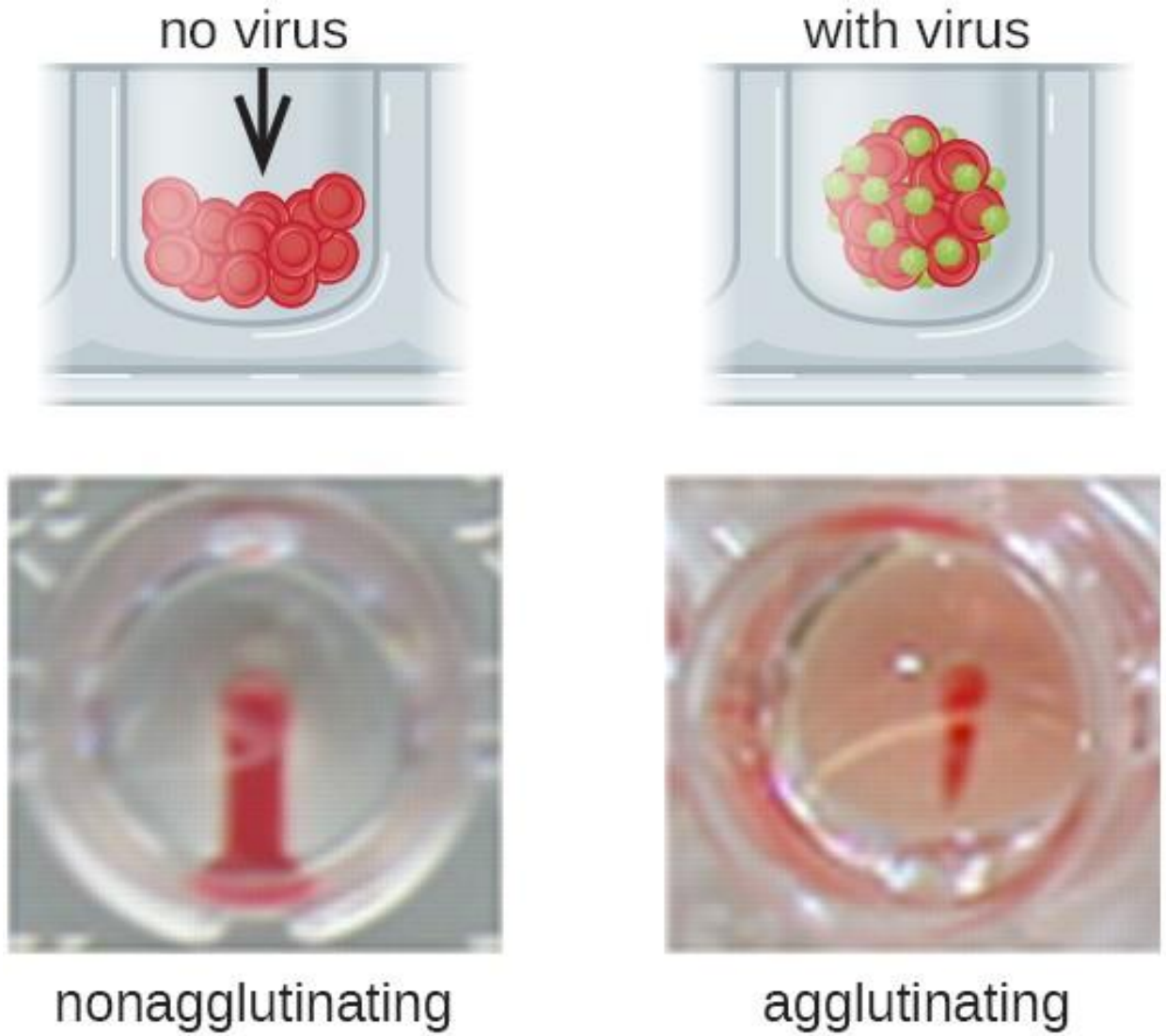


HA (+) HA (-)

In **V-bottom microwell plate**

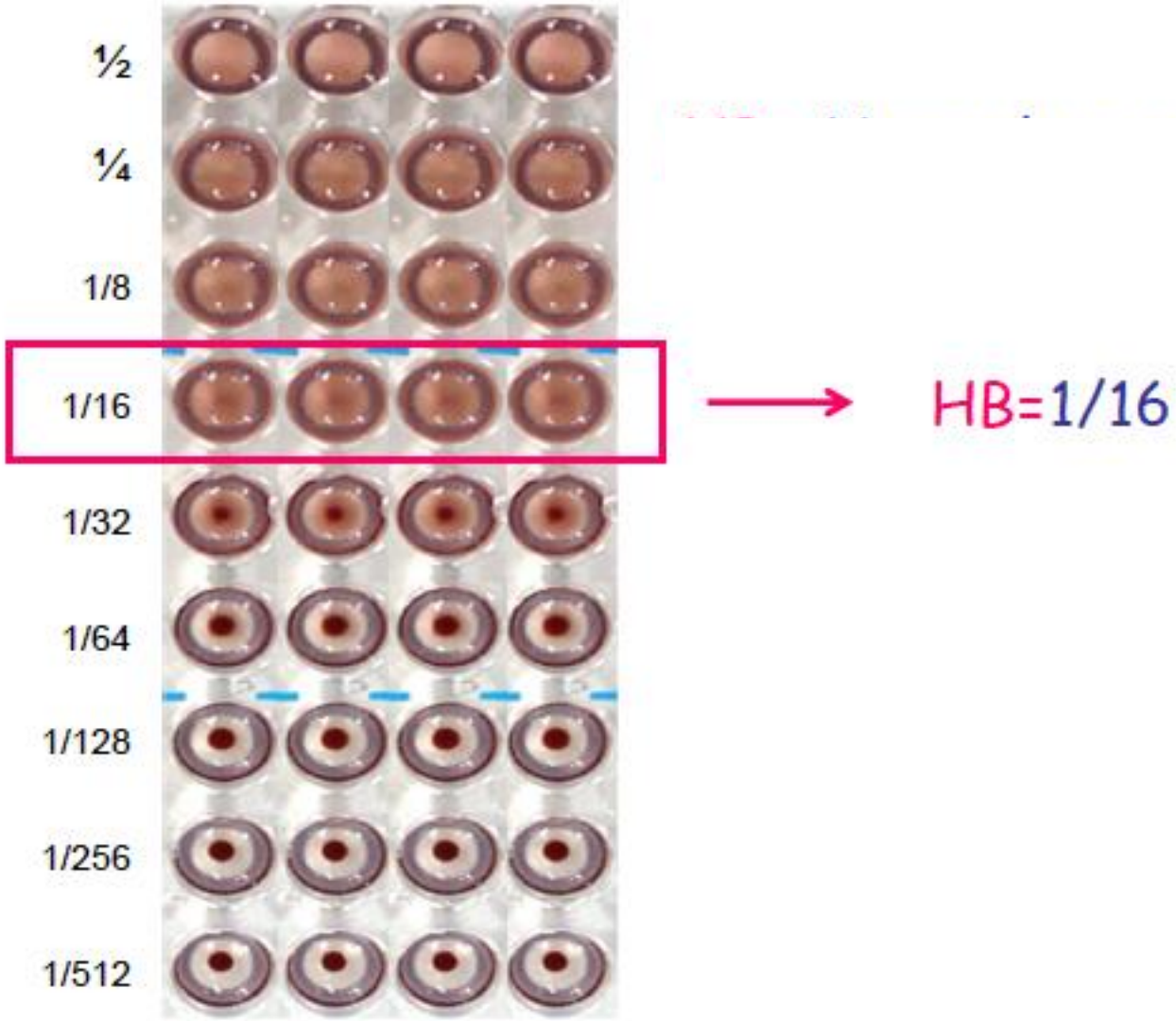


HA (+) HA (-)



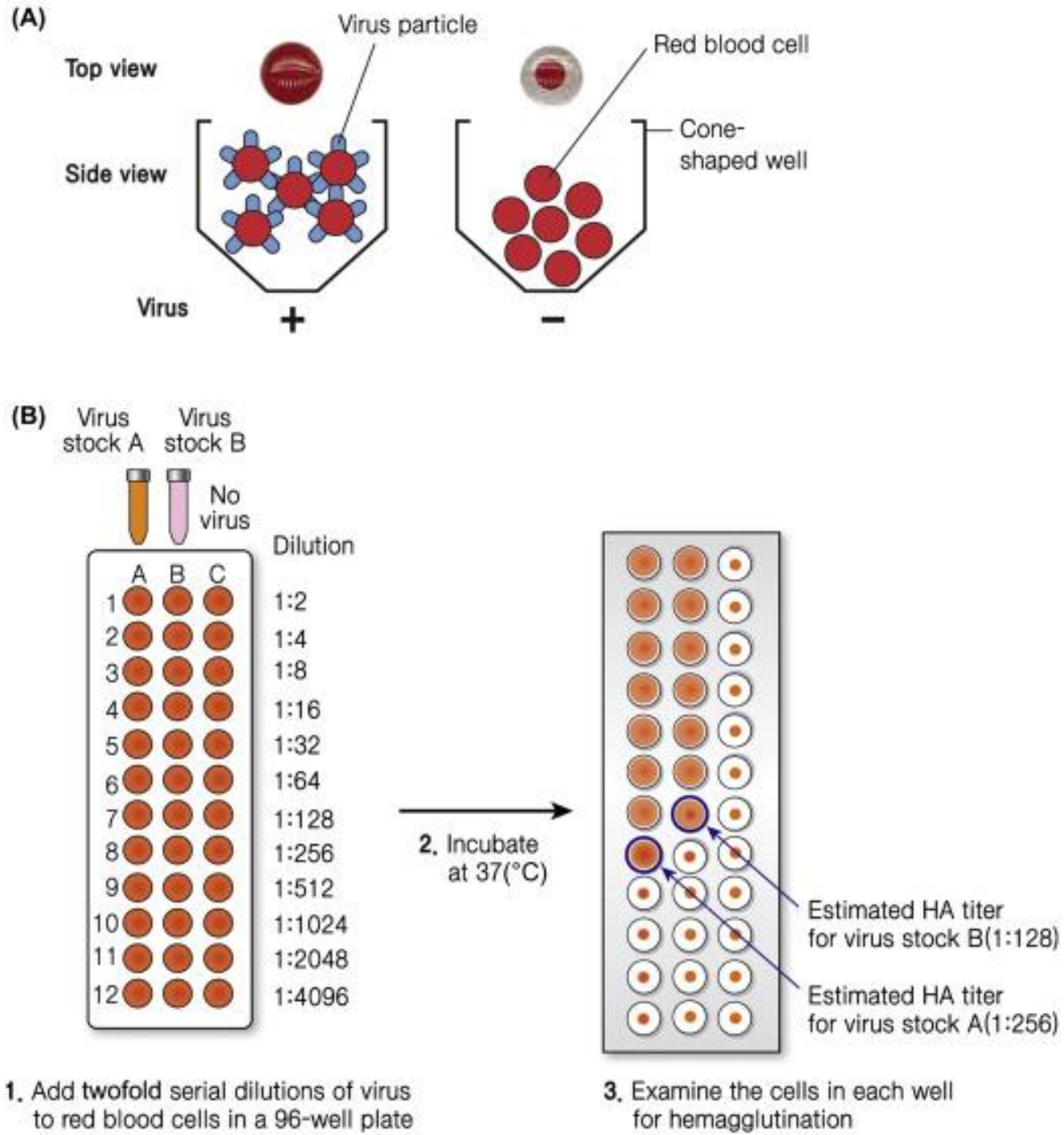
credit bottom: modification of work by American Society for Microbiology https://www.nursinghero.com/study-guides/microbiology/agglutination-assays

Determination of HA titre (HB)



HB: The last dilution step that agglutinates the same volume of erythrocytes

dilution	HA
1/2	+
1/4	+
1/8	+
1/16	+
1/32	-



Wang-Shick Ryu, Chapter 4 - Diagnosis and Methods, Editor(s): Wang-Shick Ryu, Molecular Virology of Human Pathogenic Viruses, Academic Press, 2017, Pages 47-62
https://doi.org/10.1016/B978-0-12-800838-6.00004-7.
(https://www.sciencedirect.com/science/article/pii/B9780128008386000047)

HB= The last dilution step that agglutinates the same volume of erythrocytes

4HB= 4xHB → Use for the HI test

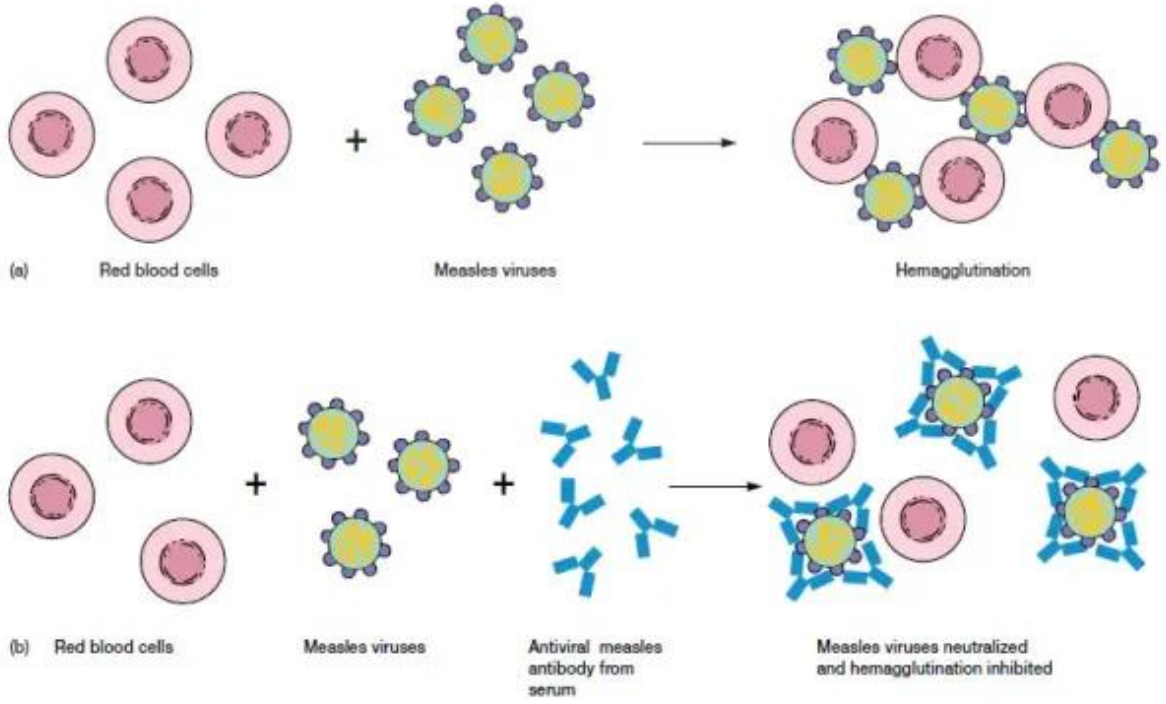
Dilution	HA	
1/2	+	
1/4	+	
1/8	+	
1/16	+	→ HB
1/32	-	

HB= 16

4HB= 4 x 1/16 = 1/4

HEMAGGLUTINATION INHIBITION ASSAY (HI)

- HI is the inhibition of the hemagglutination ability of the virus with a specific serum.
- Therefore, HI assay can be used to detect antibodies.



https://microbeonline.com/hemagglutination-inhibition-test-hai-principle-procedure-result-interpretations/

The HI test is applied for 2 purposes.

1. Identification of antigen (virus dilution method)
2. Detection of antibody in suspected serum and calculation the Ab titre in serum (serum dilution method)

1. Identification of antigen (virus dilution method)

- Suspected virus (?)
- Known serum (Ab)

- If the suspected virus and serum homologues

antigen-antibody complexes will be formed and HA does not occur

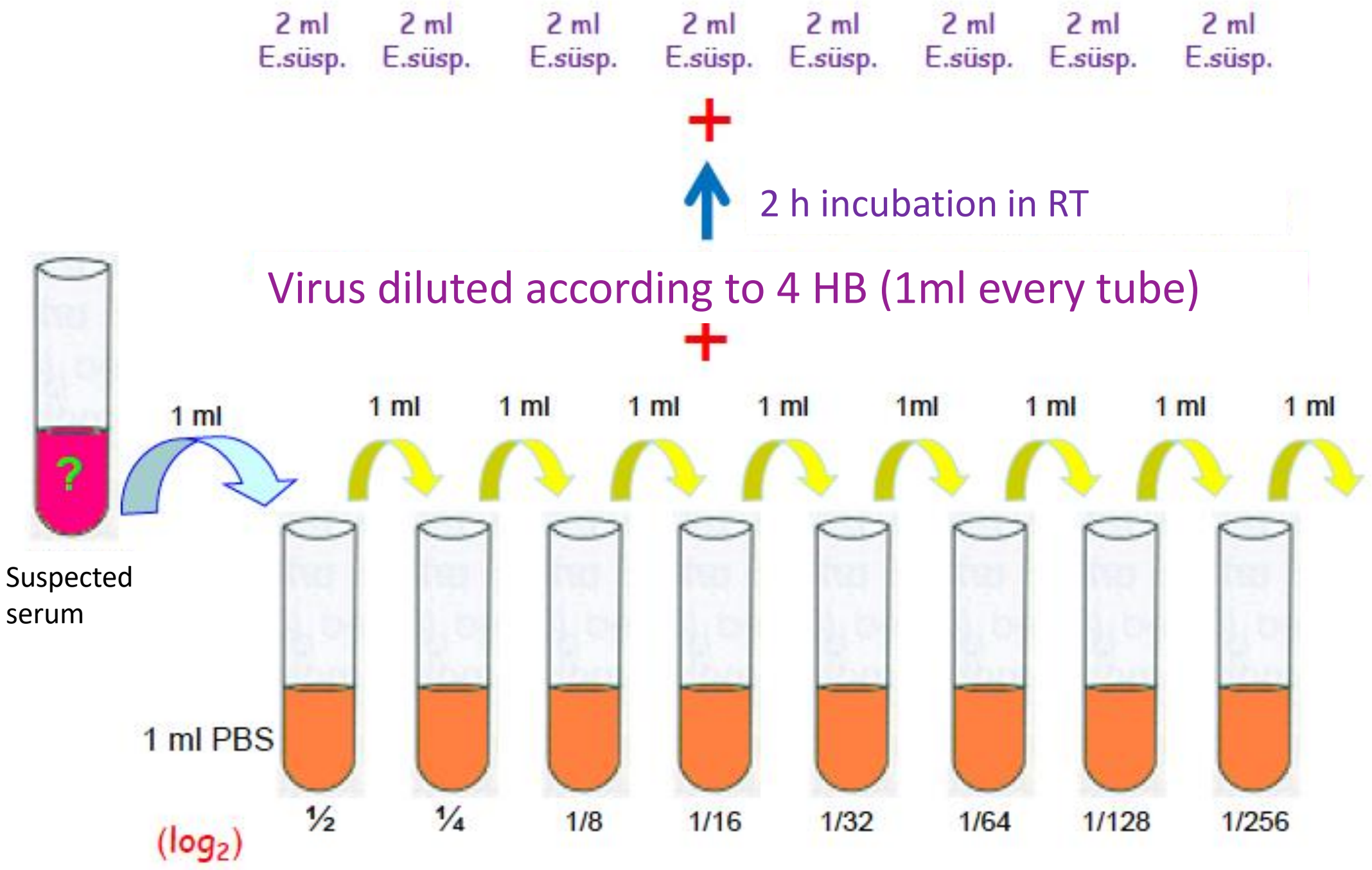
In this case, HI (+)

- If Virus(?) and serum not homologues :
- virus will attach to eritrocyte and HA occurs

In this case, HI (-)

2. Detection of antibody in suspected serum (serum dilution method)

- Known virus
- Suspected serum (?)
  - We look for Antibody


2 ml E.süsp.
2 ml E.süsp.
2 ml E.süsp.
2 ml E.süsp.
2 ml E.süsp.
2 ml E.süsp.
2 ml E.süsp.
2 ml E.süsp.
2 h incubation in RT
Virus diluted according to 4 HB (1ml every tube)
1 ml
1 ml
1 ml
1 ml
1 ml
1ml
1 ml
1 ml
1 ml
?
Suspected serum
1 ml PBS
½
¼
1/8
1/16
1/32
1/64
1/128
1/256
(log2)

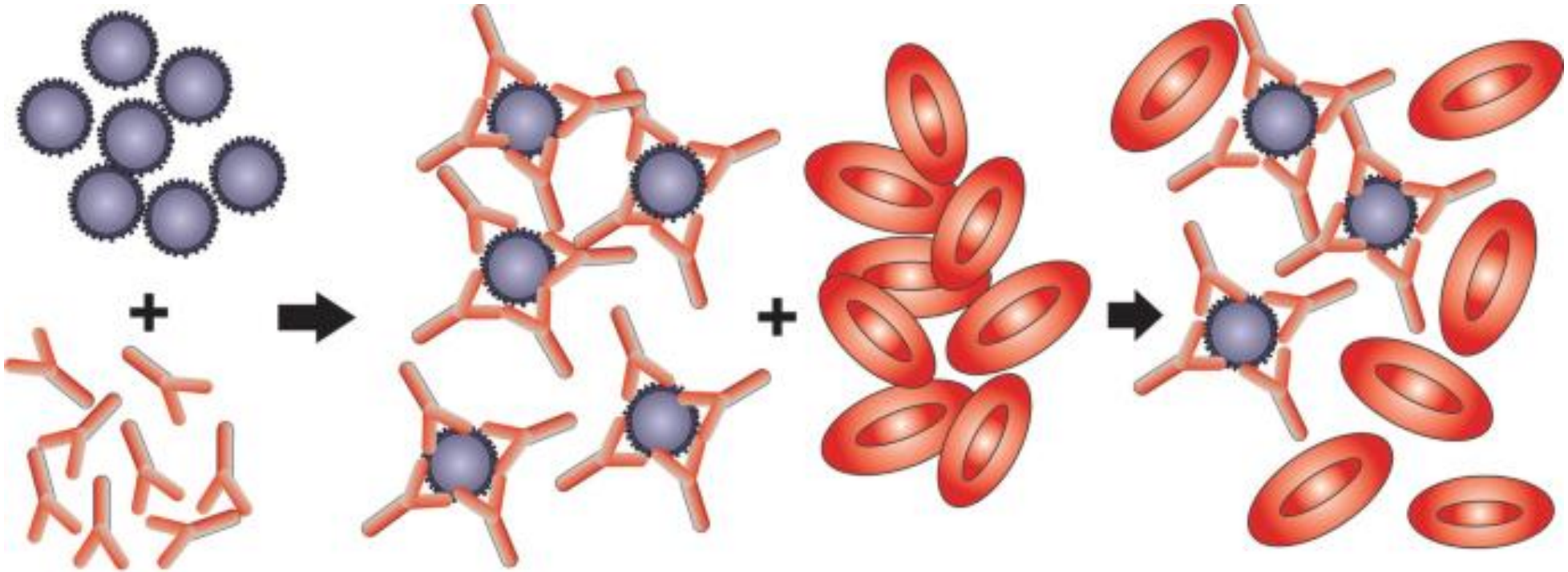
- If the suspected serum (Ab) and virus homologues

antigen-antibody complexes will be formed and HA does not occur

In this case, HI (+)

- If Serum (?) and virus not homologues :
- virus will attach to eritrocyte and HA occurs

In this case, HI (-)



1. Virus and antibody are incubated together.

2. The antibody binds the virus.

3. Red blood cells are added.

4. The antibody blocks the red blood cells from binding the virus.

Spackman, E., Sitaras, I. (2020). Hemagglutination Inhibition Assay. In: Spackman, E. (eds) Animal Influenza Virus. Methods in Molecular Biology, vol 2123. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0346-8_2

https://virology.ws/2009/05/27/influenza-hemagglutination-inhibition-assay/

Determination serum HI Titer

Sulandırma	HA	HI
1/2	(-)	(+)
1/4	(-)	(+)
1/8	(-)	(+)
1/16	(-)	(+)
1/32	(+)	(-)
1/64	(+)	(-)
1/128	(+)	(-)
1/256	(+)	(-)

Serum HI Titer:

The last serum dilution where the inhibition is seen times 4.

Serum HI Titresi: (1:16)x4= 1/4