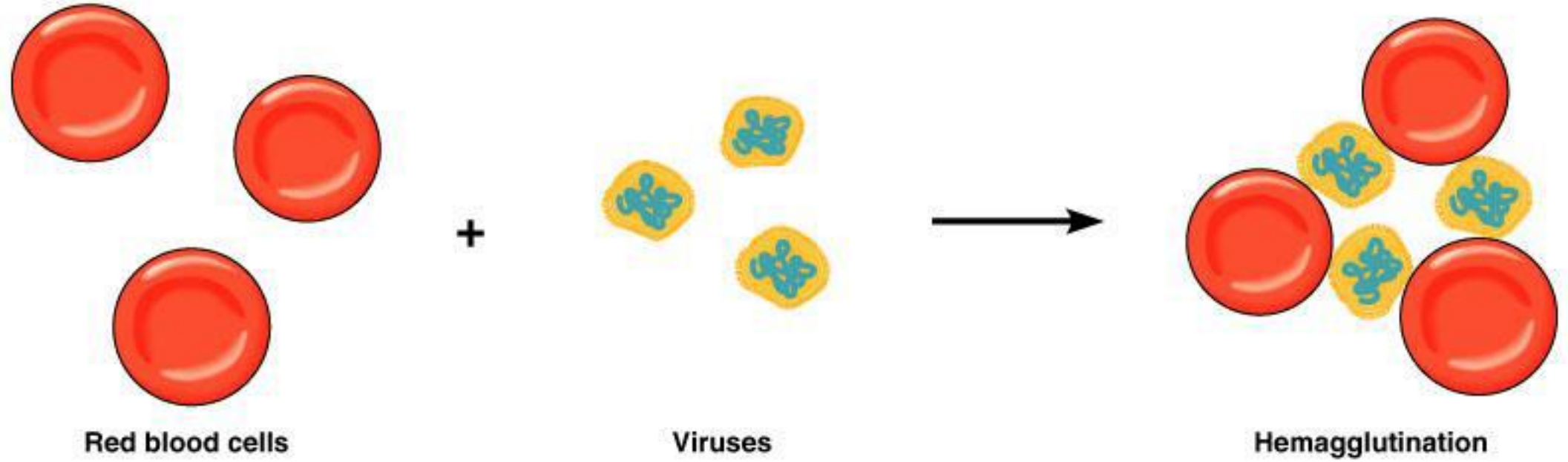
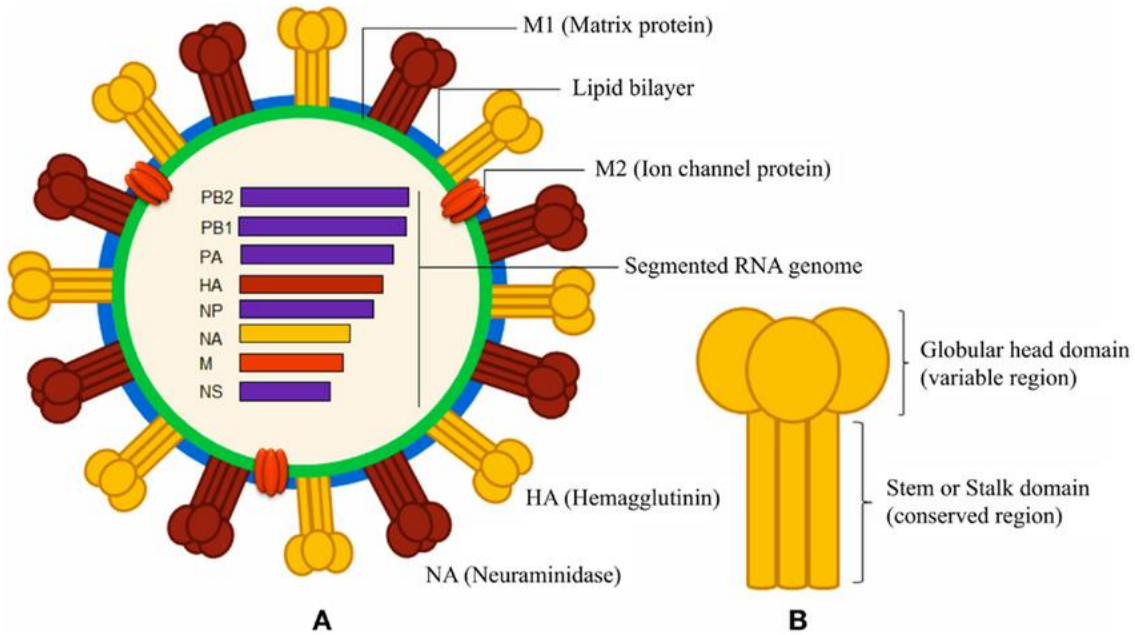
Hemagglutination (HA)

Definition: The agglutination of suspended red blood cells by **viruses**



- 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.

- 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 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

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% according to the purpose.
- -The erythrocyte suspension can be stored at + 4 °C for up to 1 month.

Hemagglutination can be performed in two ways according to purpose

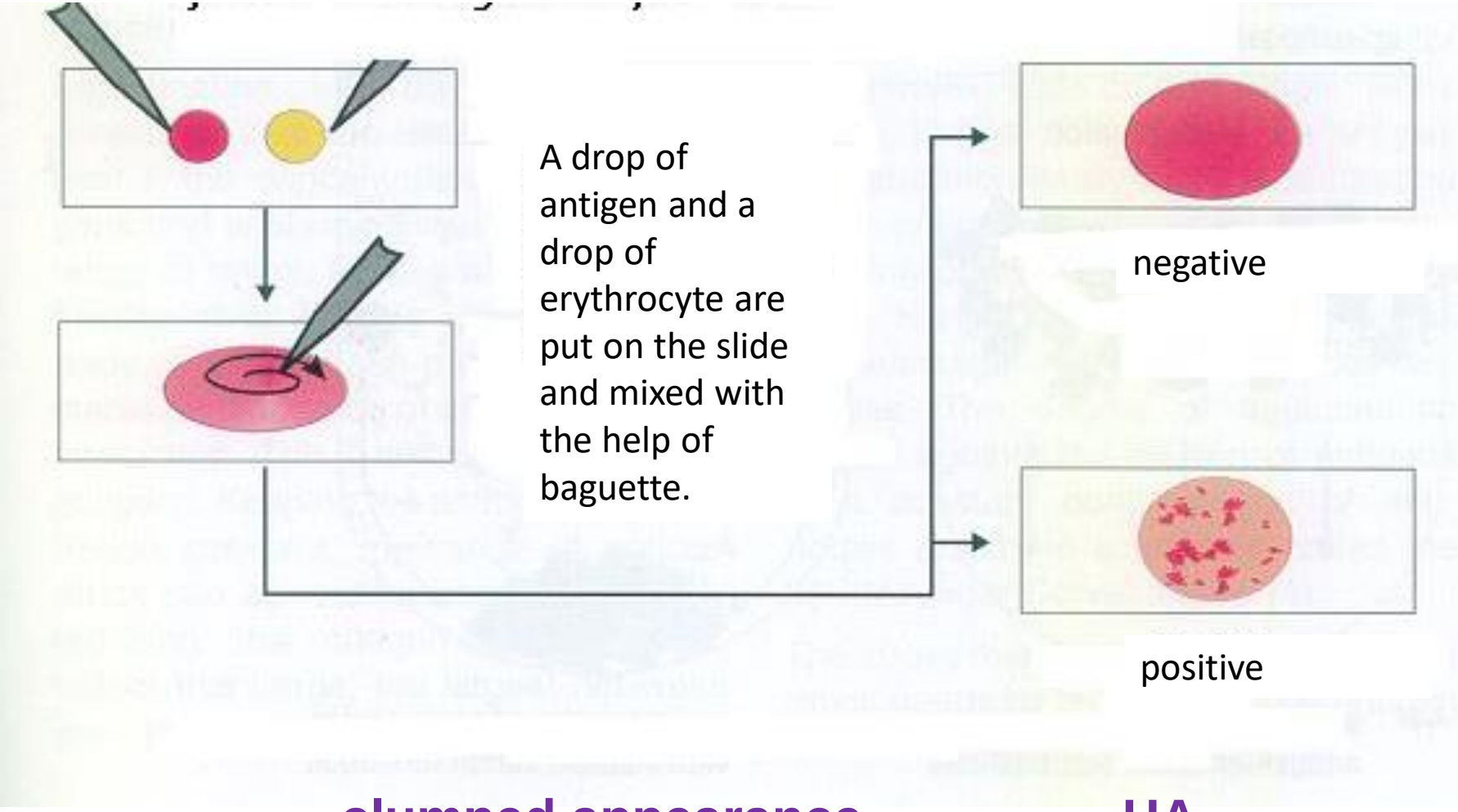
1. Rapid HA (on the slide):

- Qualitative evaluation can be done. (positive or negative)
- It is understandable whether or not the virus has HA ability and which species have erythrocytes HA.
- 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 in suspension have a clumped appearance distinct from non-agglutinated red blood cells.

clumped appearance
(+)
(-)

HA
(+)
(-)

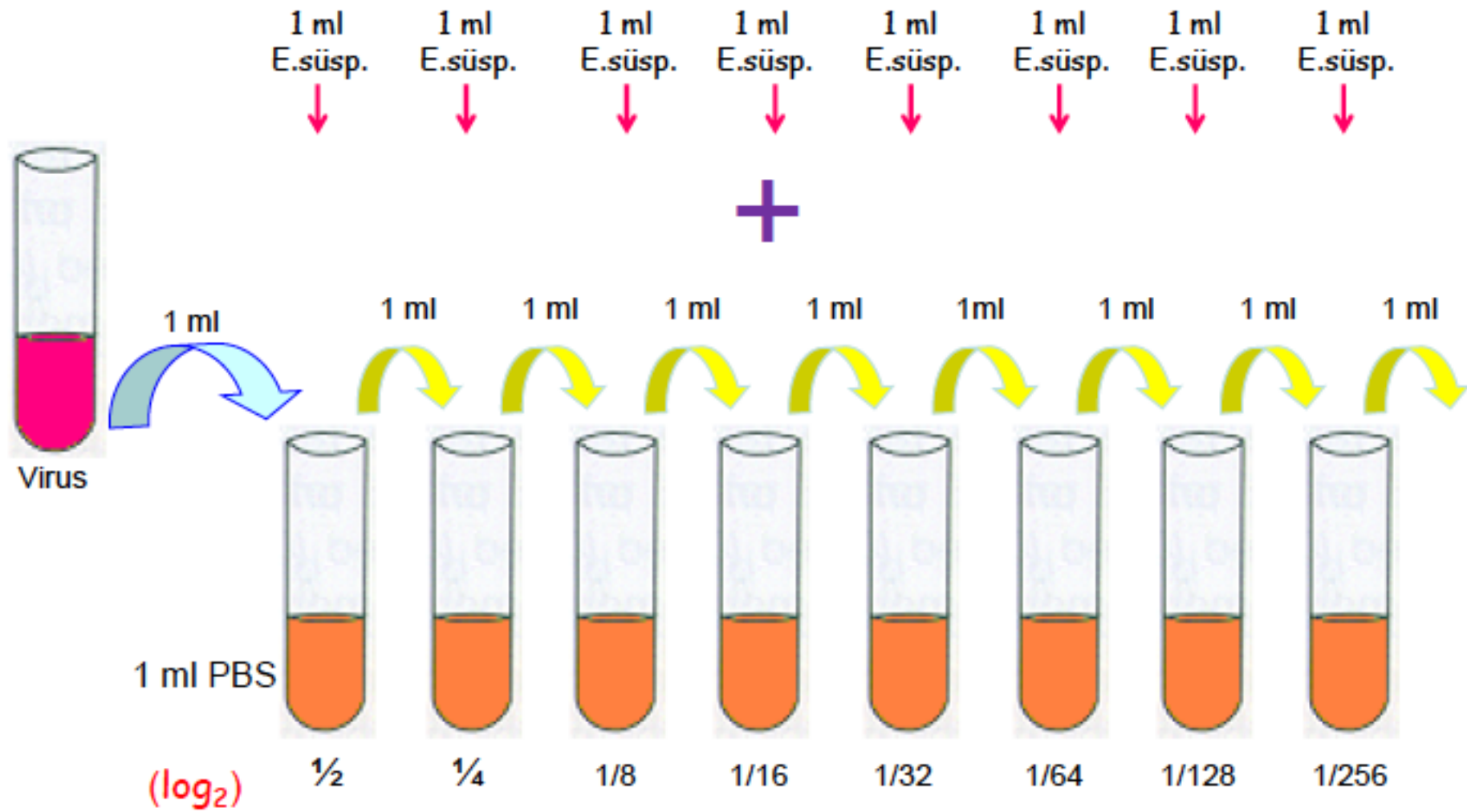
C.Staak, F.Salchow, N.Denzin : Practical Serology from the Basics to the testing, 2001.

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

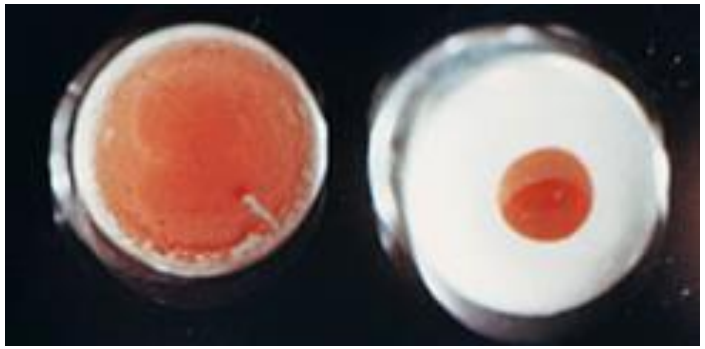
Principle;

- 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

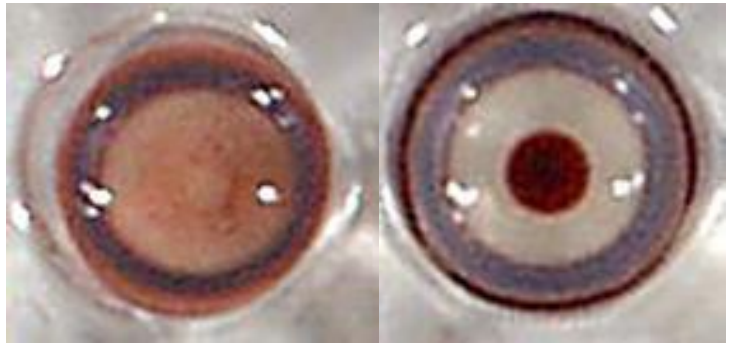
- Evaluation of HA test;
- GRID-DIFFUSE (reddish) style image HA (+)
- BUTTON (dot)-style display HA (-)

In tubes:



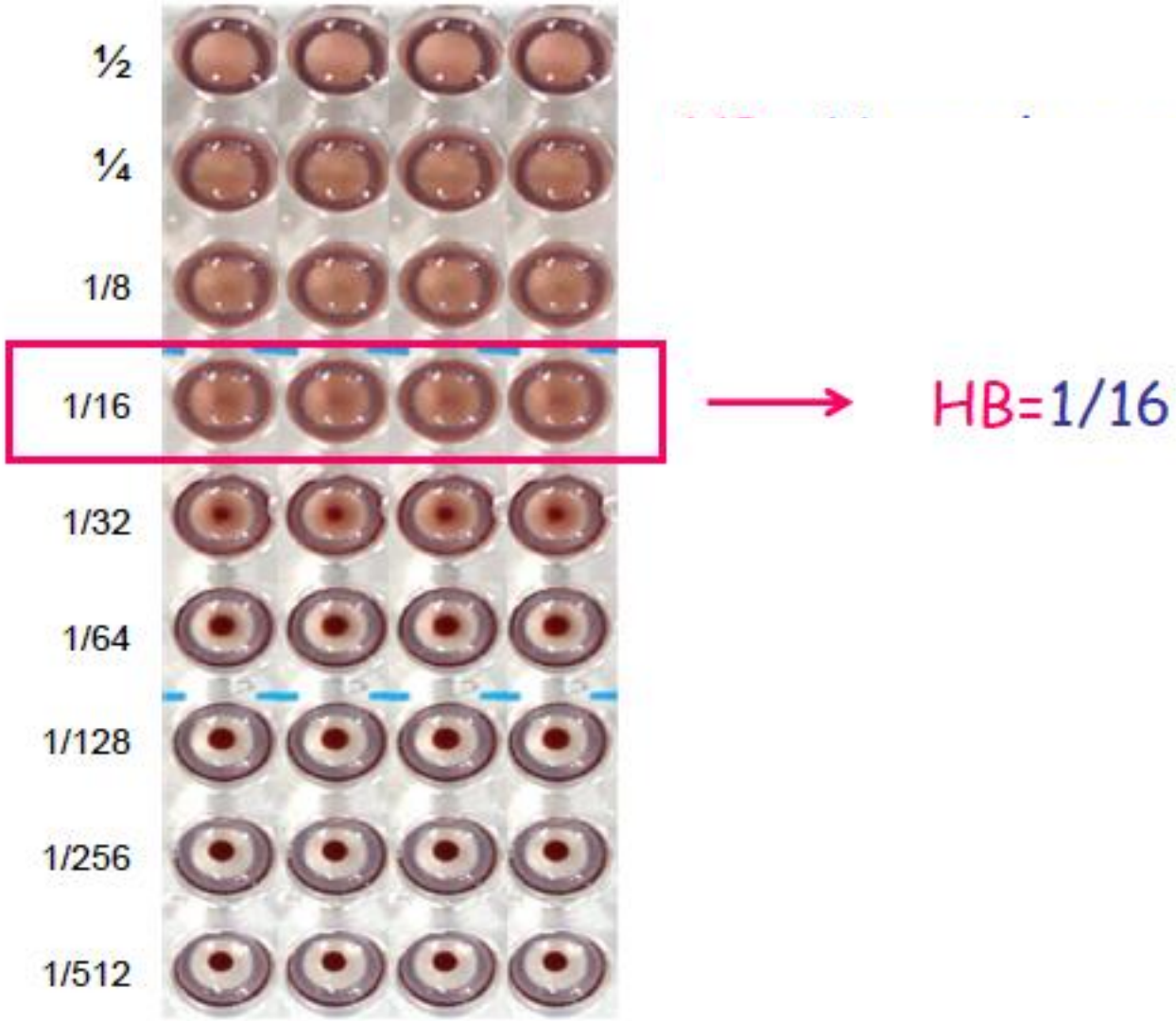
HA (+) HA (-)

In **V-bottom microwell plate**



HA (+) HA (-)

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	-

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

4HB= 4xHB → Use for the HI test

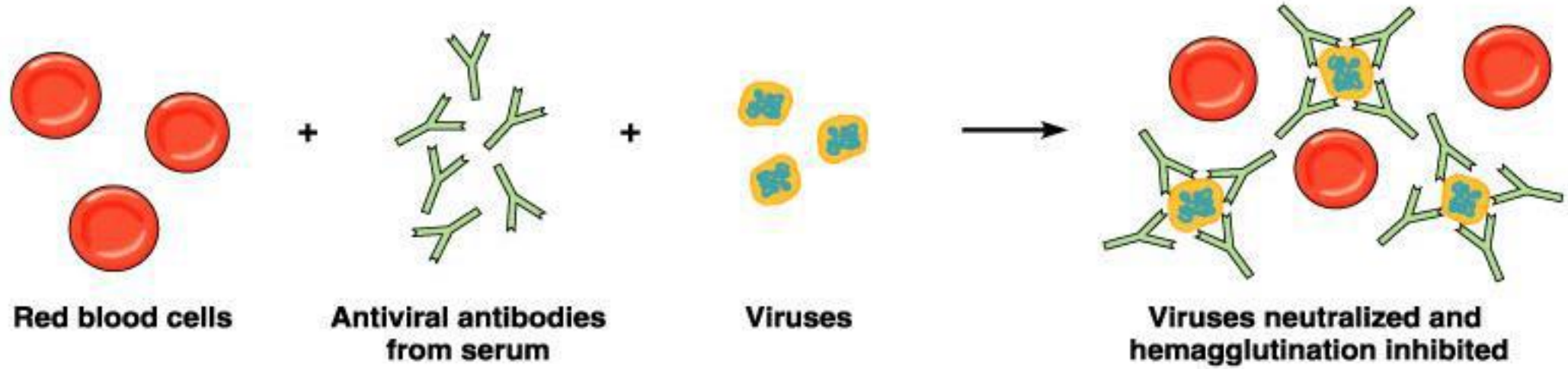
Dilution	HA
1/2	+
1/4	+
1/8	+
1/16	+ → HB
1/32	-

HB= 16

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

HAEMAGGLUTINATION INHIBITION ASSAY (HI)

- HI is the inhibition of the hemagglutination ability of the virus with a specific serum.



- **(b) Viral hemagglutination test to detect antibodies to a virus. These viruses will normally cause hemagglutination when mixed with red blood cells. If antibodies to the virus are present, as shown here, they neutralize and inhibit hemagglutination.**

- Therefore, HI assay can be used to detect antibodies.

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

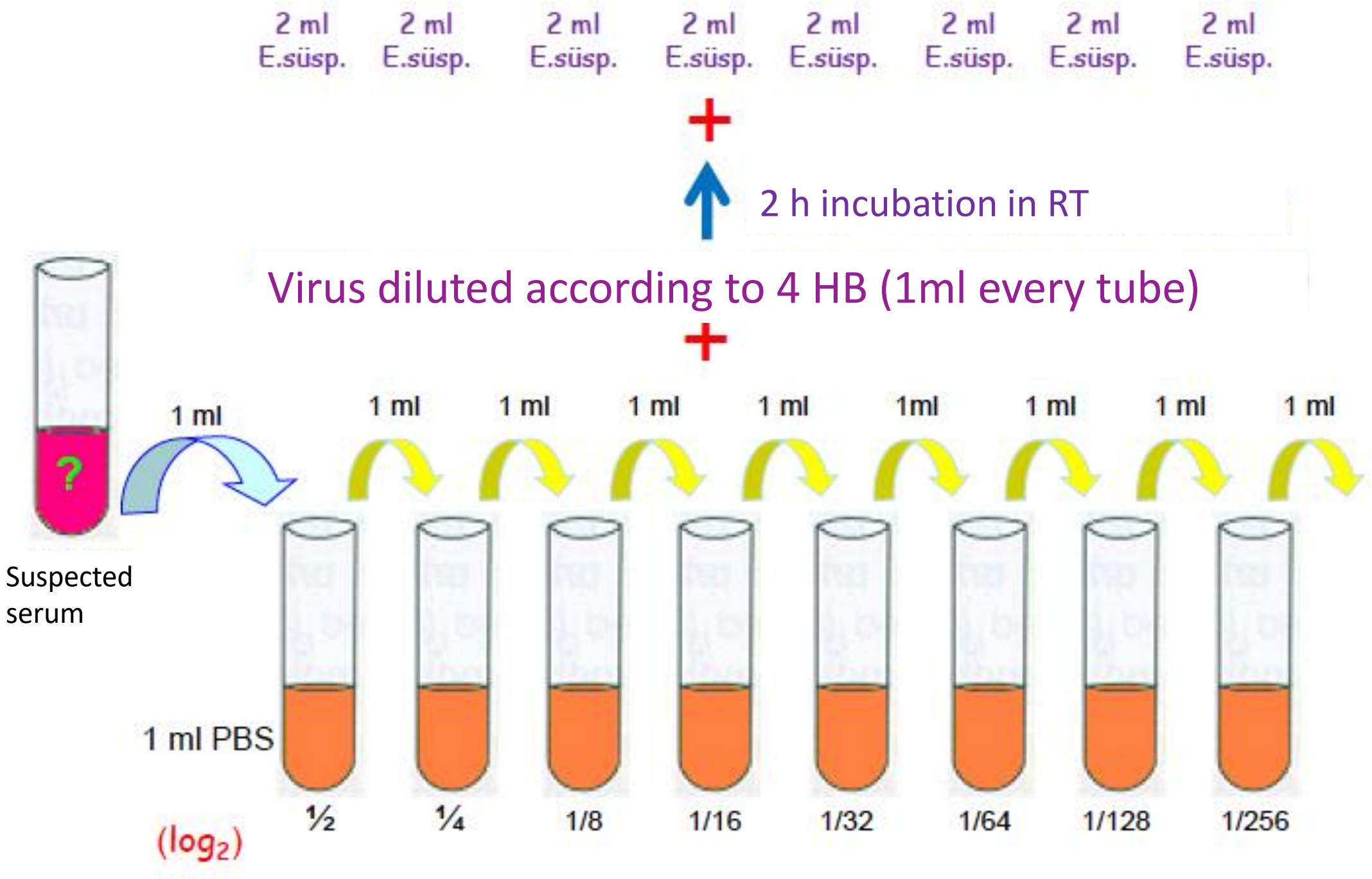
In this case, HI (+)

- 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)

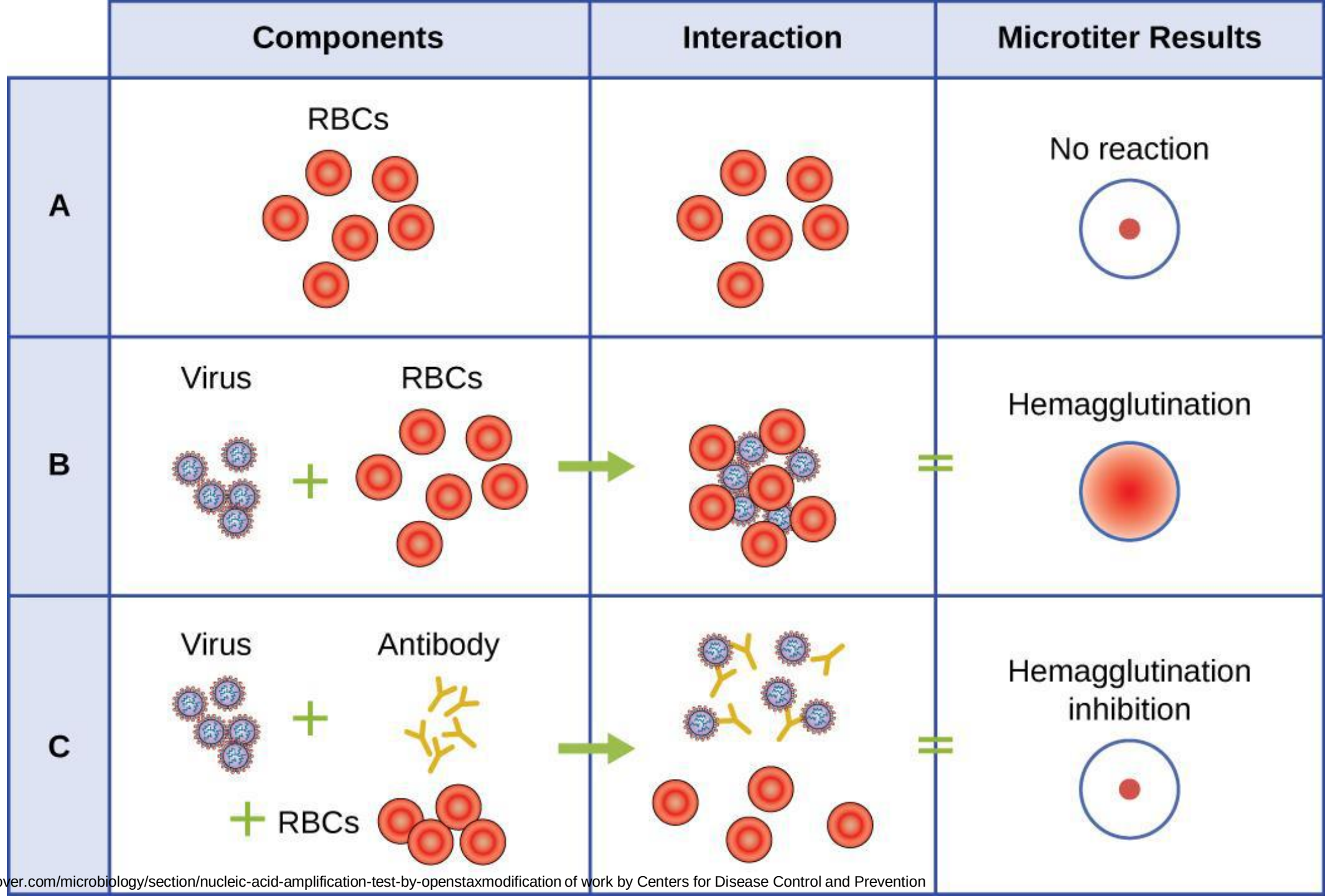
- If the suspected serum (Ab) and virus homologues

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

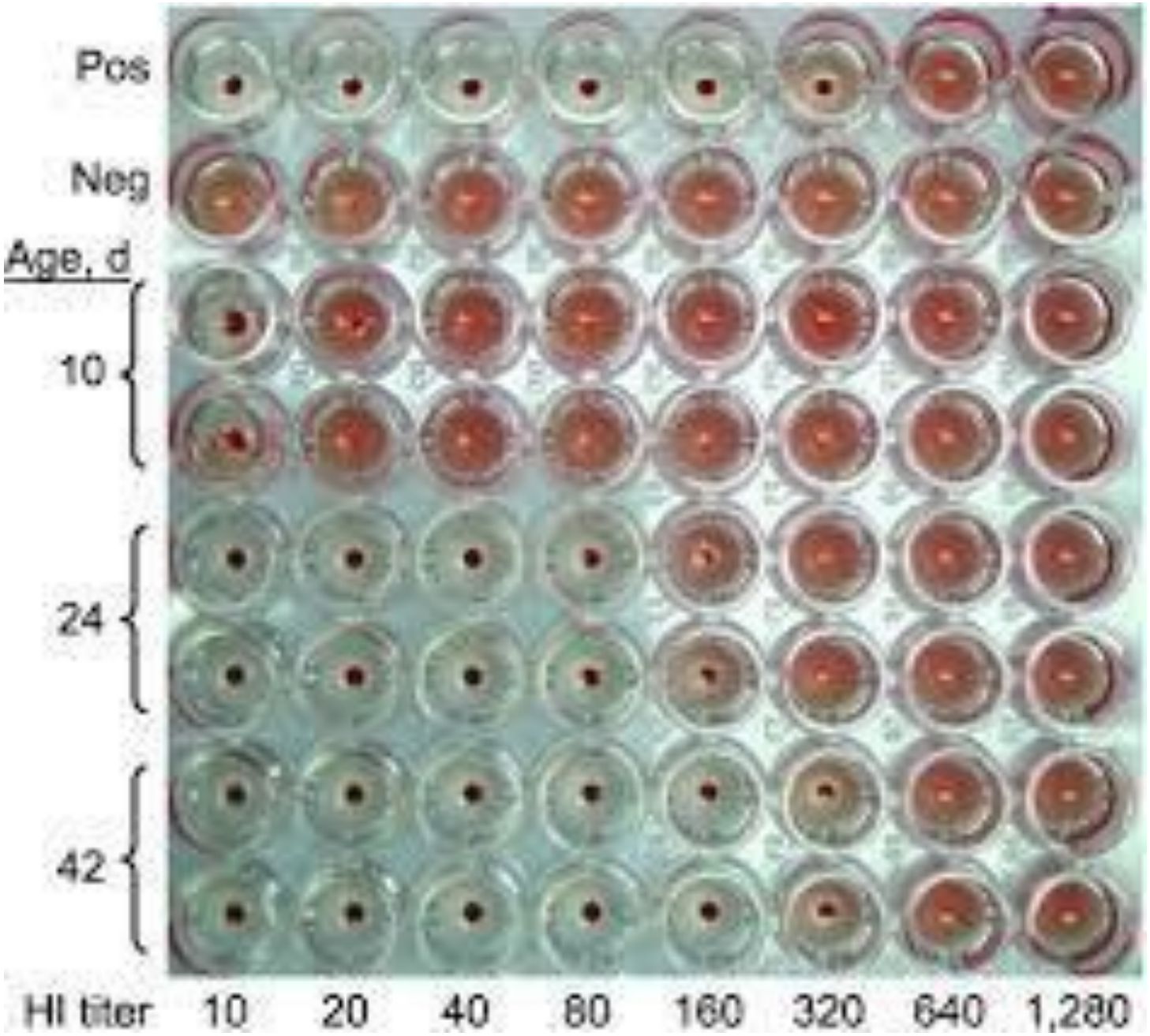
In this case, HI (+)

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

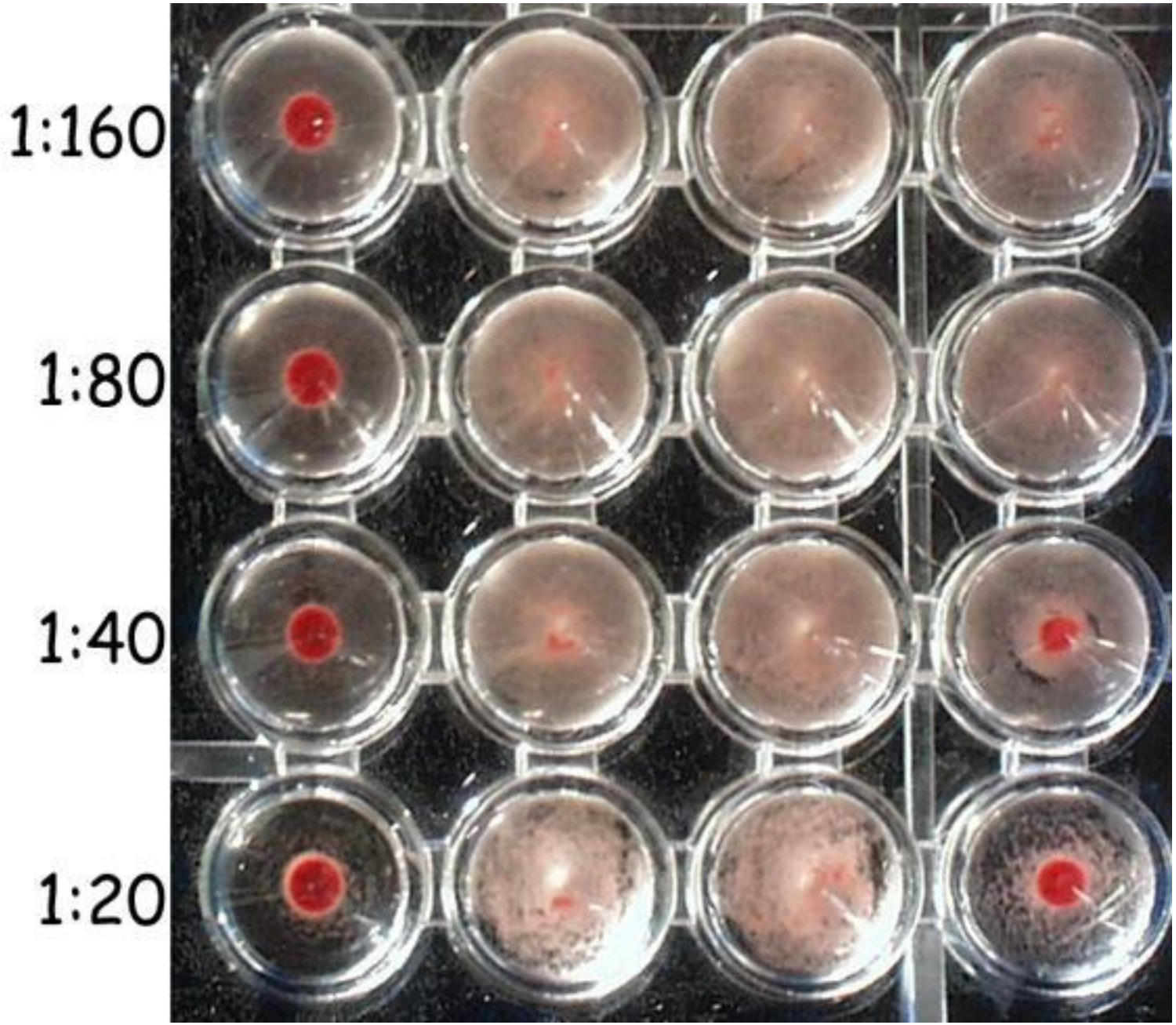
In this case, HI (-)



https://www.quizover.com/microbiology/section/nucleic-acid-amplification-test-by-openstaxmodification of work by Centers for Disease Control and Prevention



Perinatalpandemic(H1N1) 2009 infection, Thailand.DulyachaiW, MakkochJ, RianthavornP, ChangpinyoM, PrayangprechaS, PayungpornS, TantilertcharoenR, KitikoonP, PoovorawanY -EmergingInfect. Dis. (2010)


1:160
1:80
1:40
1:20

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