College of Medicine
Al-NAHRAIN UNIVERSITY

Practical Virology Lab-6- Virus Titration

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Assay of viruses Titration:

❑Biological

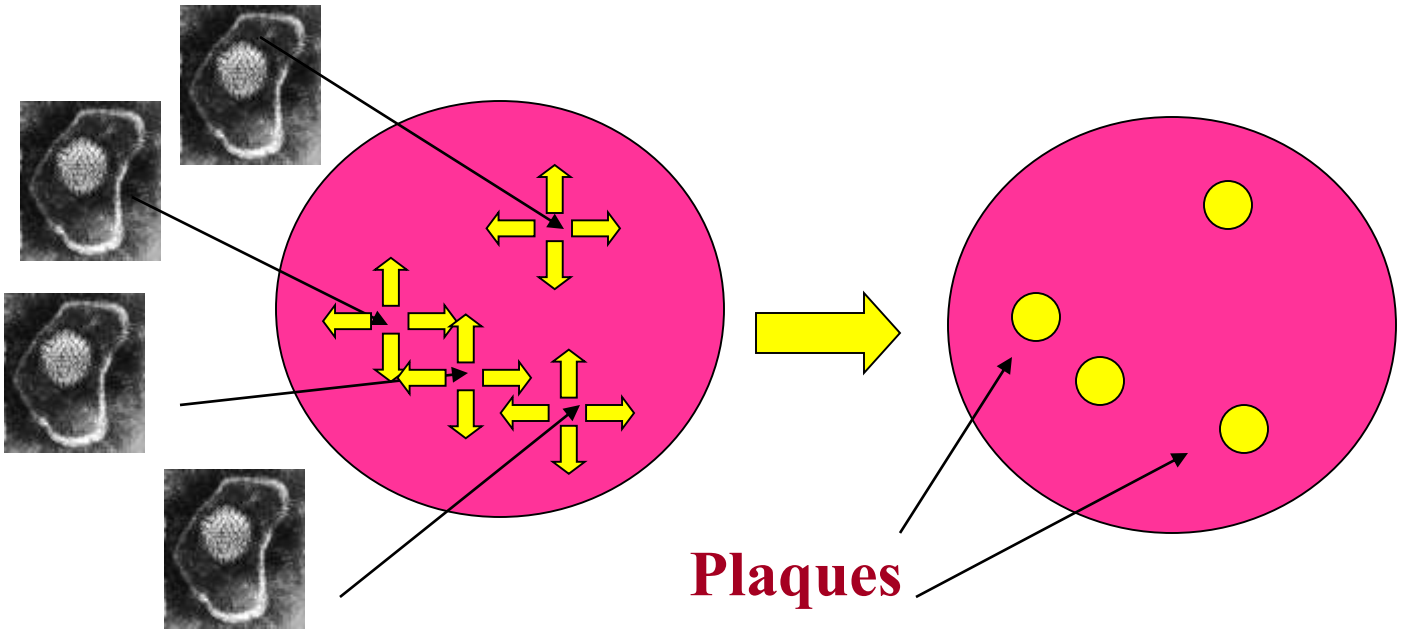
A. Plaque assay
B. Endpoint Method: $TCID_{50}$, EID_{50}, & LD_{50}.

❑Physical

A. Hemagglutination.
B. Direct particle count.

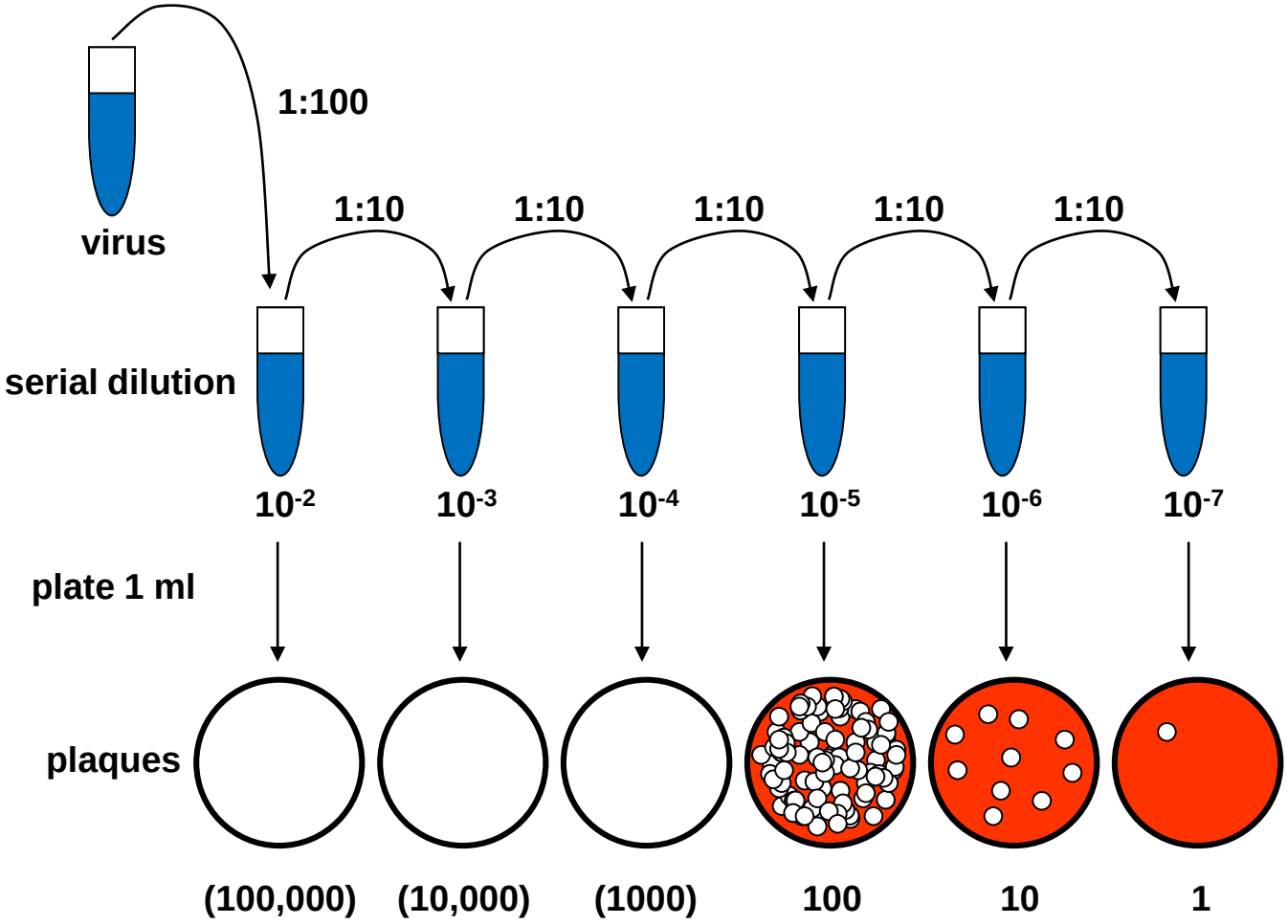
Virus Titration by Biological Methods

A- Plaque Assay



Count plaques(plaque forming unit/ml)

Procedure:

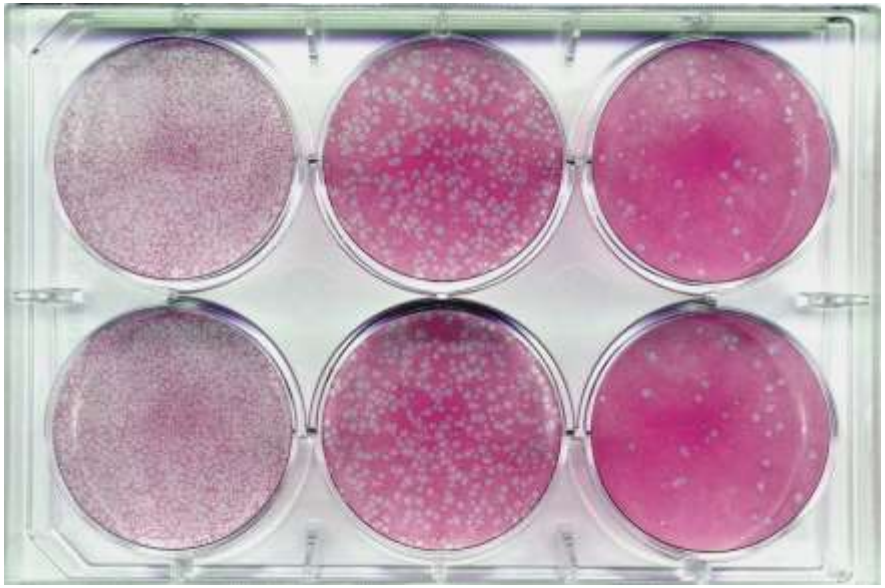


Titer = 1 x 10^7 pfu/ml

Plaque assay applications:

- **Identification of the virus by very specific anti-sera.**

- **Quantitation of the virus titer in the sample (Plaque forming unit) = PFU**



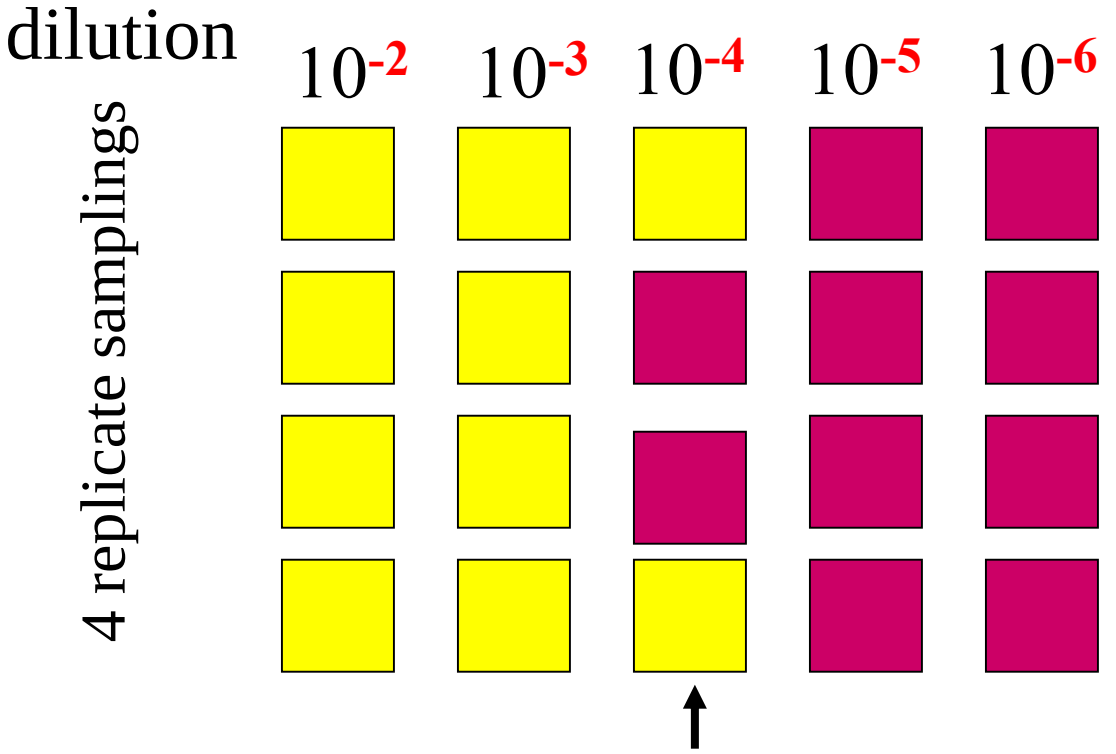
Plaque assays used to quantitate a viral stock.

Virus Titration by Biological Methods

B- TCID 50 Tissue Culture Infectious Dose 50%:

1. Prepare a monolayer of cell culture in flat bottomed wells of a microtiter plate.
2. Do serial dilution of the sample that contain the virus (e.g.10 fold dilution).
3. Inoculate each dilution in a well.
4. Incubate at 37˚C &daily examine for the appearance of CPE.
5. Determine the infectivity of the virus using Karber method, (TCID 50 equals to the reciprocal of dilution that causes CPE in 50% of the cells in the monolayer in the well).

Procedure:


dilution
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
4 replicate samplings
$TCID_{50} = 10^{4}$
50% Tissue culture infectious dose

Endpoint Method: TCID50, EID50, & LD50.

- **$TCID_{50}$: is defined as that reciprocal of dilution of virus per unit volume required to infect & produce CPE in 50% of the cell cultures inoculated.**

- **The EID_{50}: is defined as the reciprocal of that dilution of virus per unit volume that results in death (or other observable endpoint) in 50% of the inoculated eggs.**

- **The LD_{50} : is defined as the reciprocal of that dilution of virus per unit volume that will kill 50% of the inoculated animals.**

Factors that influence the LD_{50}

- The age of the animal.
- The health status of the animal.
- The strain of the animal.
- The route of inoculation.
- The strain and passage of the infecting virus.
- The size of the inoculums.
- Smaller dilution interval & the larger the number of animals per experimental group, the greater the accuracy of the results obtained.

Virus Titration by Physical Methods:

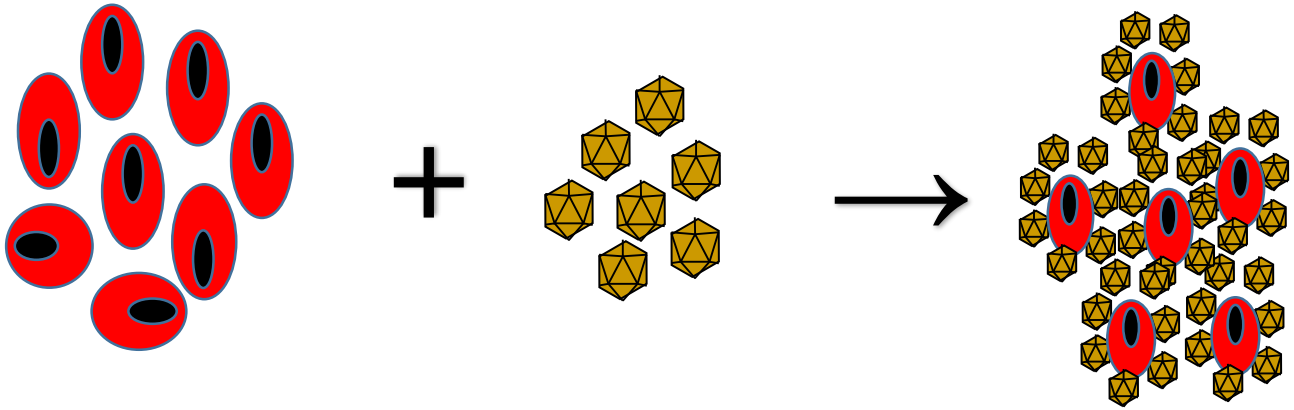
A. Hemagglutination.

- **Hemagglutination (HA) test.**
  - **Biological phenomena.**
  - **Virus titration.**

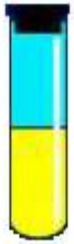
- **Hemagglutination Inhibition (HI) test.**
  - **Serological test.**
  - **Antibody titration**

B. Direct particle count.

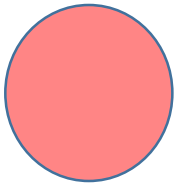
Virus Titration by Physical Methods:
A. Haemagglutination (HA) test.



RBC suspension



HA virus



Diffuse Pattern

HA test procedure:

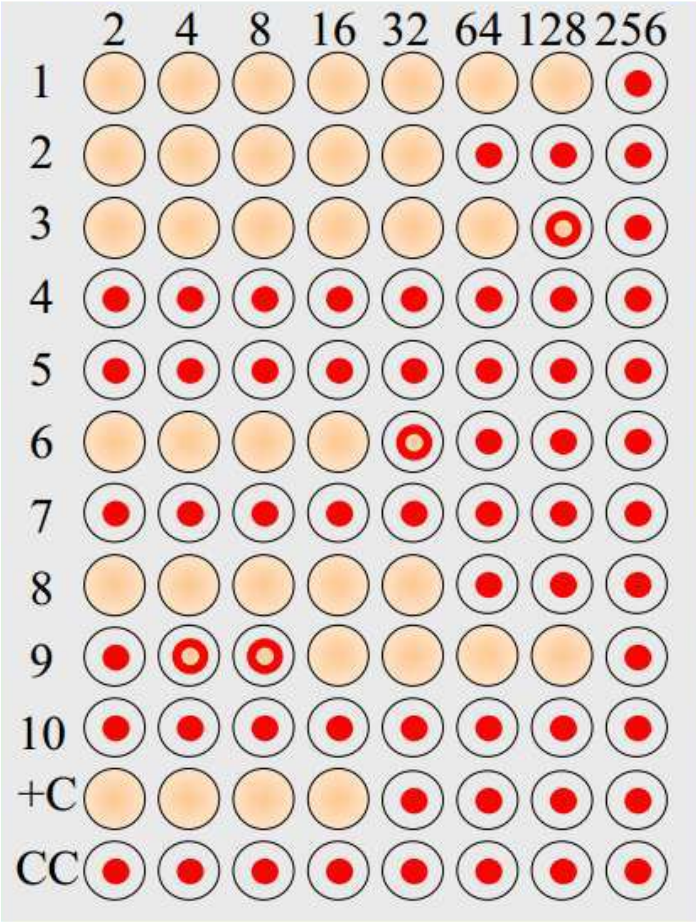
Materials	well No.									
(ml)	1	2	3	4	5	6	7	8	9	10
Saline	**0.45**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
Virus	**0.05**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**-**
Dilution	**1:10**	**1:20**	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**1:2560**	**con**
0.5%RBC	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**

incubate the plate at room temperature for 30 to 60 min.

Check the agglutination.

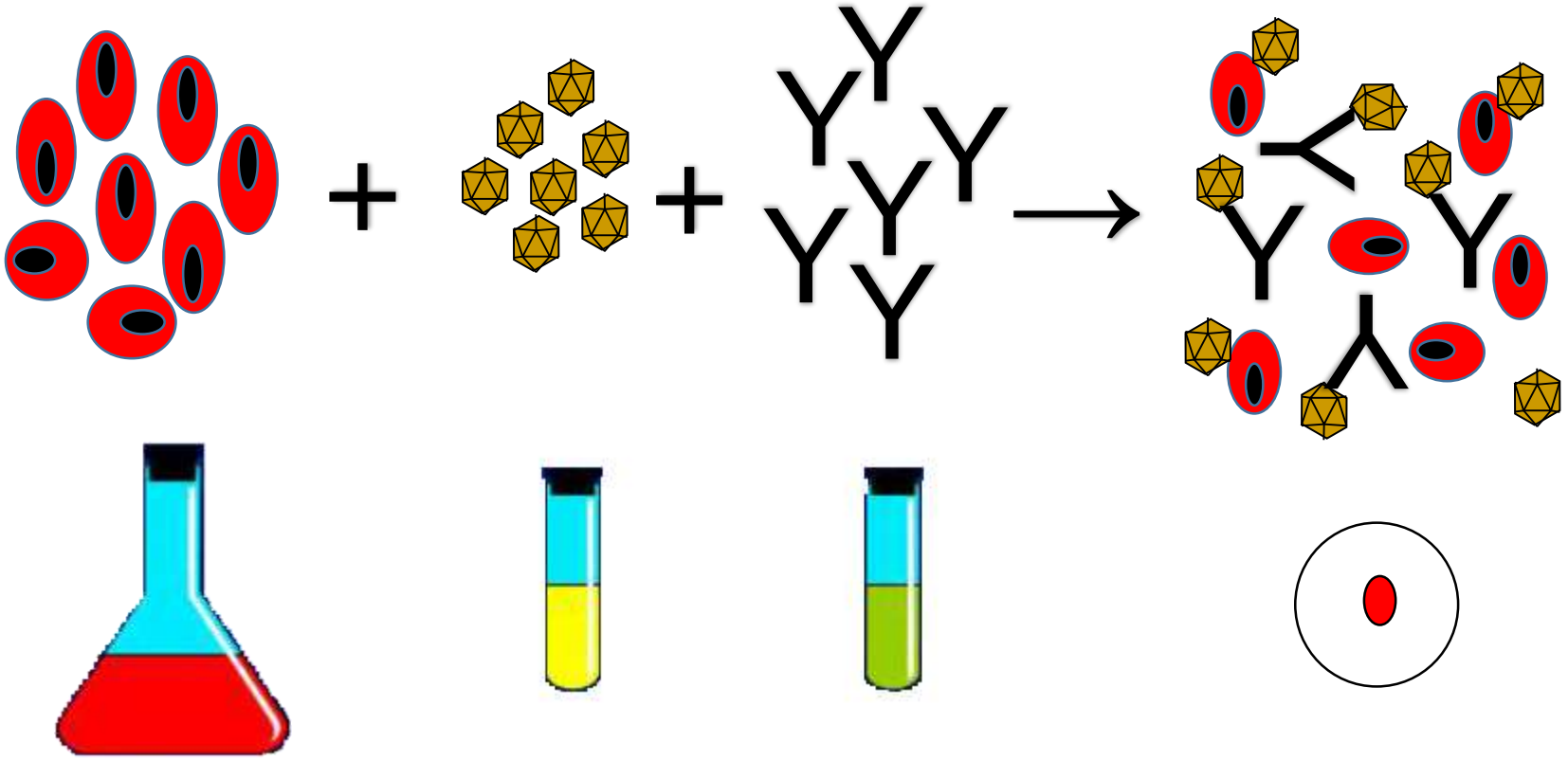
Interpretation of HA test:

HA titer = the reciprocal of highest dilution of virus giving complete HA.



Row No.	Endpoint
1	1:128
2	1:32
3	1:64
4	Neg.
5	Neg.
6	1:16
7	Neg.
8	1:32
9	1:128
10	Neg.

Haemagglutination inhibition (HI) test:

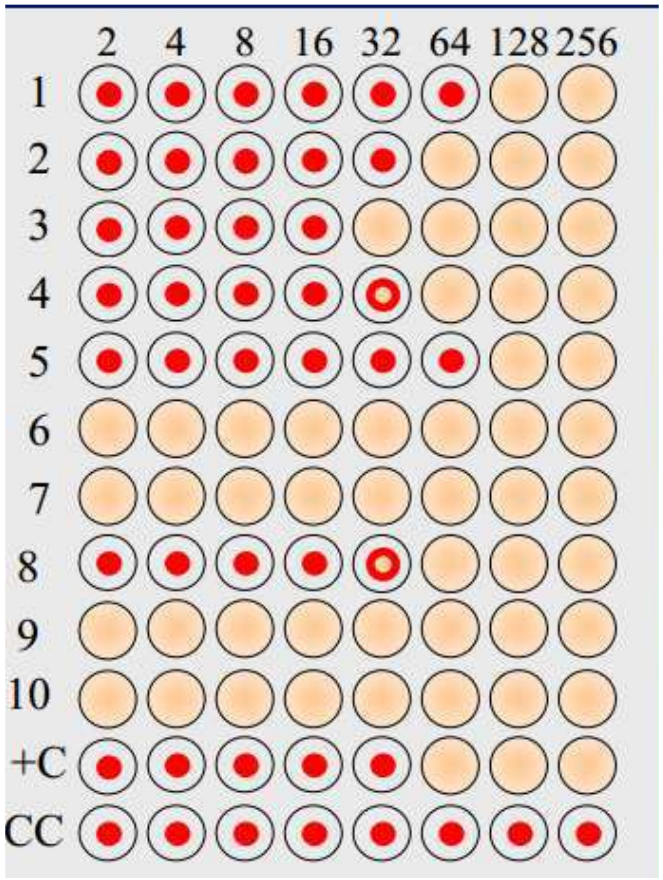


HI test procedure:

Materials	well No.									
(ml)	1	2	3	4	5	6	7	8	9	10
Saline	**0.9**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
Serum	**0.1**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**-**	**0.25**	**-**
Dilution	**1:10**	**1:20**	**1:40**	**1:80**	**1:160**	**1:320**	**1:640**	**-**	**-**	**-**
Virus	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**-**	**-**
0.5%RBC	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**-**	**0.25**
Mix evenly incubation at 37C for 1h										
Result	**-**	**-**	**-**	**+**	**++**	**+++**	**++++**	**++++**	**-**	**-**

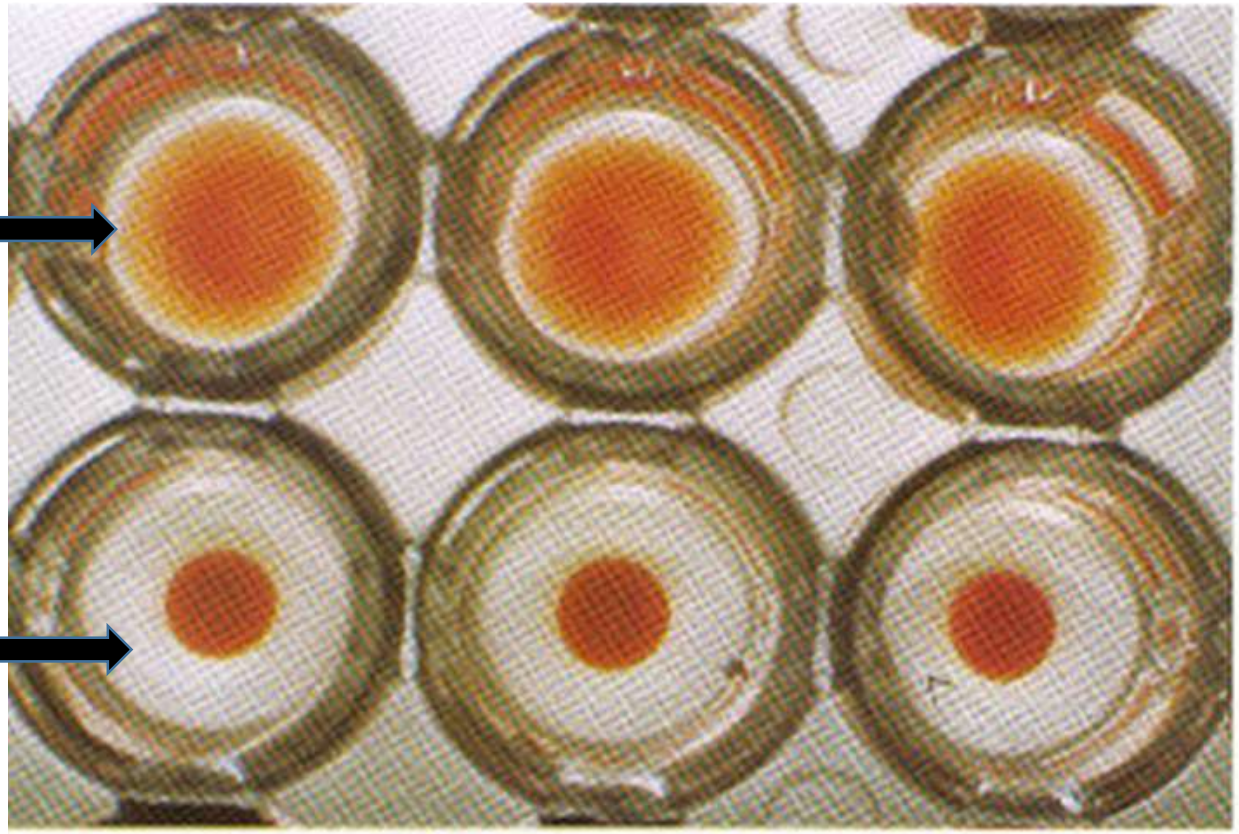
Interpretation of HI test:

HI titer is the reciprocal of the highest dilution of the patient's serum which shows complete inhibition of agglutination.



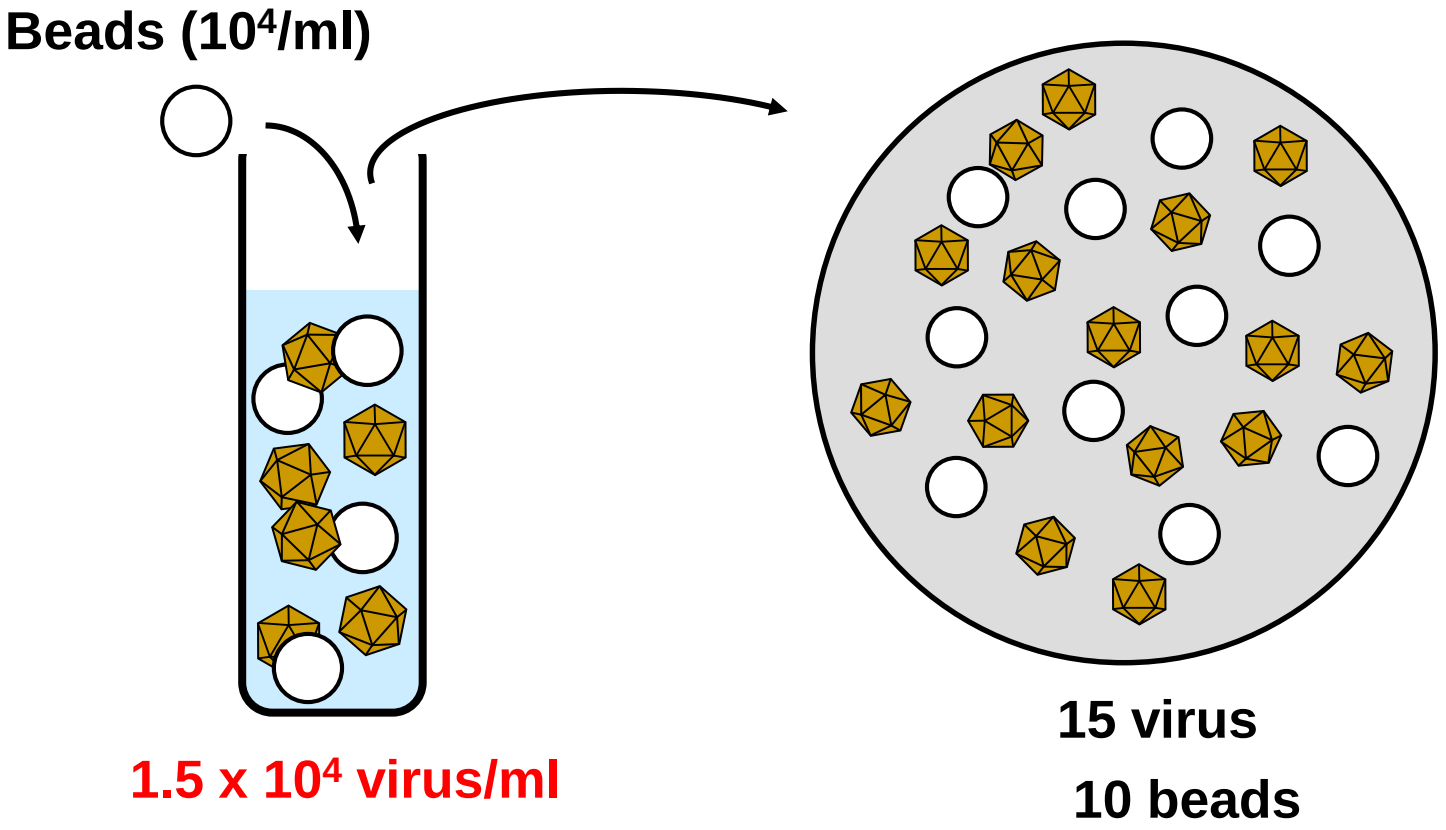
Row No.	Endpoint
1	1:64
2	1:32
3	1:16
4	1:32
5	1:64
6	Neg.
7	Neg.
8	1:32
9	Neg.
10	Neg.

Haemagglutination Vs. Haemagglutination Inhibition Analysis of the Results:

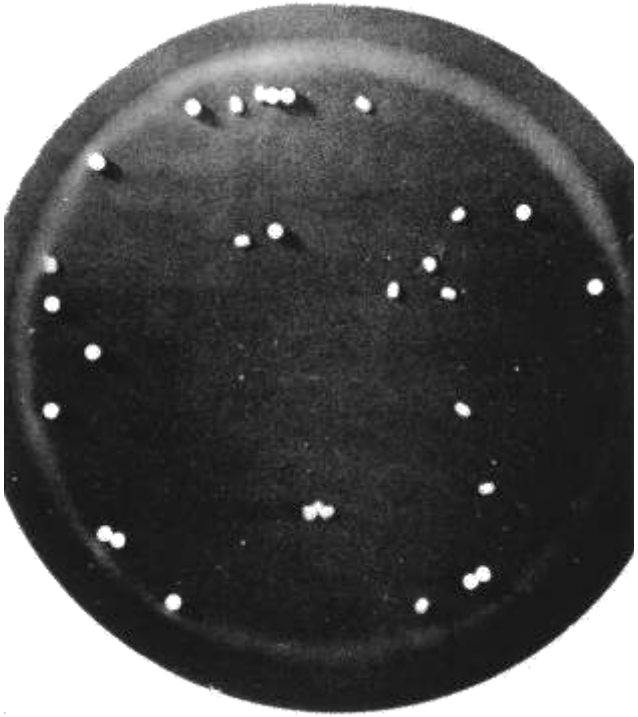


Virus Titration by Physical Methods:

B. Direct particle count.



Direct particle count



Direct electron microscopic particle count. An electron micrograph of a spray droplet containing 15 latex beads (spheres) and 14 vaccinia virus particles (slightly smaller, brick-shaped particles). (From Fields Vriology (2007) 5th edition, Knipe, DM & Howley, PM, eds, Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia Fig. 2.8)

SOME POINTS TO REMEMBER

Infectivity:

- Not every released particle is infectious.

Assays:

- Detect every particle (e.g. electron microscope)
- Detect infectious particles only (e.g. plaque assay)


THANK
YOU