SAWA UNIVERSITY

# VIROLOGY

## preservation& titration of Virus


Sawa University — جامعـــــة ســـــــاوة الاهلية

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Department of Medical Laboratories — قسم تقنيات المختبرات الطبية

. Three :Stage — المرحلـــــــــــة الثالثة


الجانب العملي — محاضرة رقم 5.. ...

Practical — Lecture No.5 ..


تدريسي المادة : م. د. سيف علي

# Virus Preservation

The ability to store viruses for long periods of time with minimal loss of viability is critical. Viruses exhibit a wide variety of structural and chemical differences, but, in general, their infectivity may be destroyed by:-

1- Degradative enzymes that destroy nucleic acids

2- Detergents that solubilize the lipid-containing envelopes

3- Temperatures higher than about 50 degrees C

4- Chemicals that breakdown capsid proteins.

# Methods of preservation

The methods which viruses may be preserved for long periods of time are similar to those employed for other microorganisms, these methods include:-

1- Cryopreservation in liquid nitrogen,

2- Lyophilization (freeze-drying), and

3- Storage at low temperature (-70˚C) in mechanical freezers

# Virus Titration

# Virus Titration

Determine the amount of virus particles in a sample . There are multiple methods to measure the amount of virus in the sample, such as Real-Time (RT) PCR,Western Blot, ELISA, and flow cytometry. These methods utilize the amount of viral DNA, RNA, or proteins to quantify the virus. However, these methods do not measure the actual biological activities of the virus.

**Viroids (infectious whole live viral particles)** or it is a quantitative estimation of infection Viral titration: The content of infectious viruses in any viral suspension can be assayed. Make a series of dilutions of the viral suspension in a phosphate-buffered saline solution and then check on the over the days following the injection to see evidence of the effect on virus reproduction.

Quantitative titrations: In these methods, the number of verions (whole live viral particles) is estimated infectious

# Unspecified quantitative titrations:

This type of assay does not record the number of live infectious viral particles in the syringe, but only what the type of virus .

It is inaccurate and is used only for viruses that do not form documents or work as a decimal string of dilutions of the virus are then infected into several replicates from cell cultures, chicken embryos or animals

# ❖ IMMUNOLOGICAL TECHNIQUES

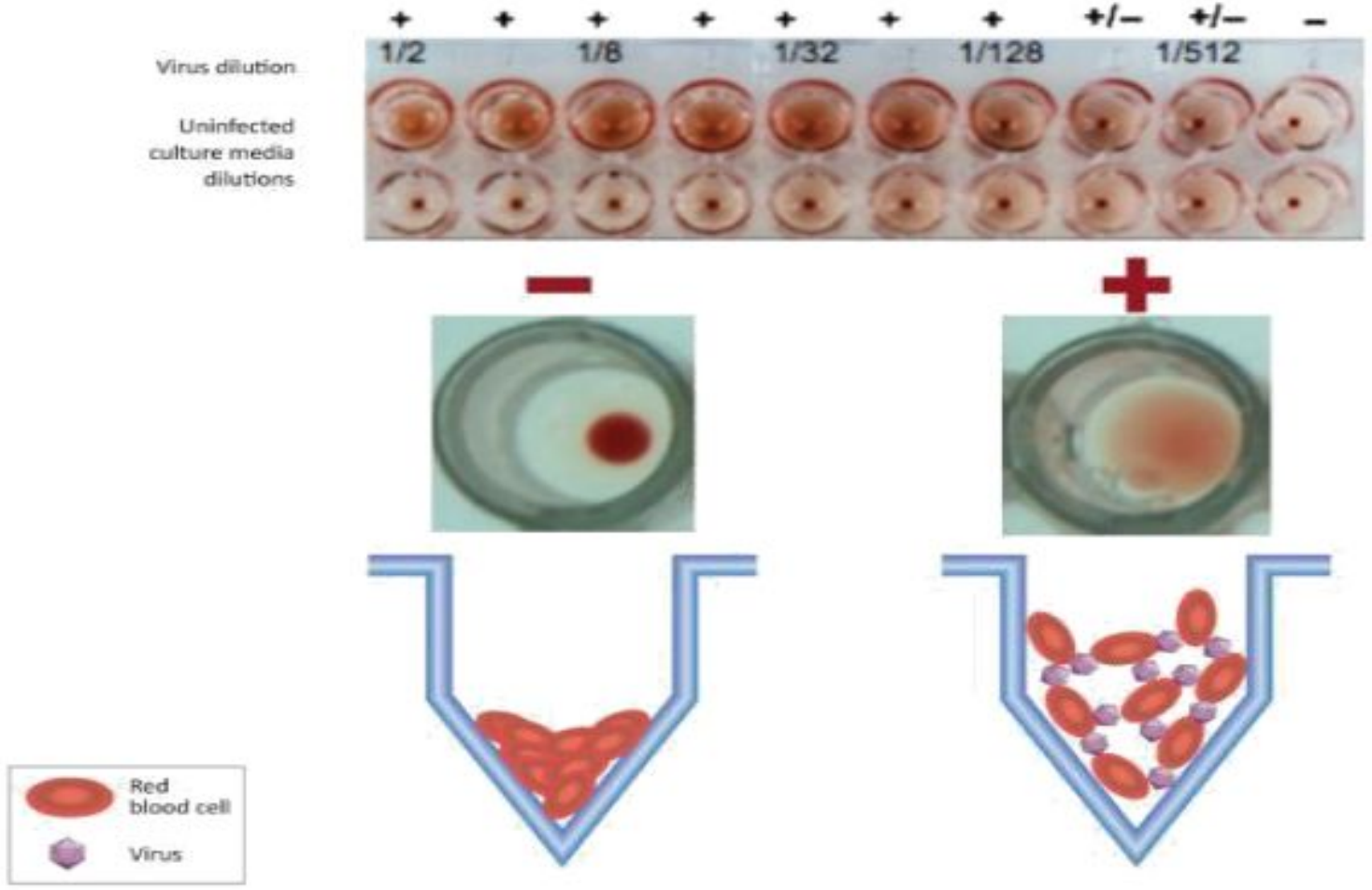
## Hemagglutination Assay :_

Some viruses bind to red blood cells (RBCs). Hemagglutinating viruses bind to sialic acid residues on the RBCs. A single virion can bind to several different RBCs, and an RBC can be bound by multiple virions to form a large network, or web, of cell and virus that is easily visualized.

The HA is fast and does not require either instrumentation or extensive training.

It is done by preparing serial dilutions of a virus sample. An aliquot of each dilution is added to RBCs in a microtiter plate well or test tube. One well contains RBCs and saline (negative control) and another contains a known positive reference sample of virus. The samples are gently mixed and allowed to sit at room temperature.

In the negative wells the RBCs will slide down to form a tight button at the bottom of the tube. In positive wells the RBCs and virions will bind to each other to form a mesh of cells on the bottom of the tube. The reciprocal of the highest dilution of virus that give a positive HA is the HA titer.


+ + + + + + + +/– +/– –
Virus dilution
1/2
1/8
1/32
1/128
1/512
Uninfected culture media dilutions
Red blood cell
Virus

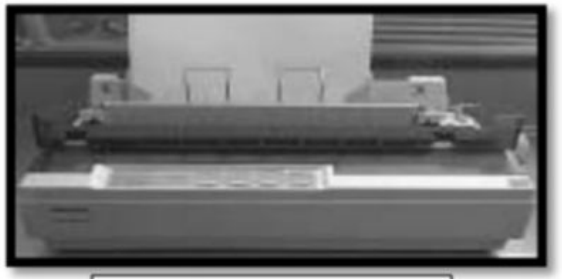
# Enzyme-Linked Immunosorbent Assays (ELISA)

ELISA is a commonly used technique for the determination of antibodies or antigens.

• ELISAs are routinely used in scientific research, veterinary medicine, environmental and agricultural applications, and in healthcare.

• There are three main types of ELISA; direct, indirect and competitive. • Often the antigens of blood borne pathogens such as HIV or hepatitis B (HBV) are detected with such systems. Neither HIV nor HBV proliferates in standard cell cultures.

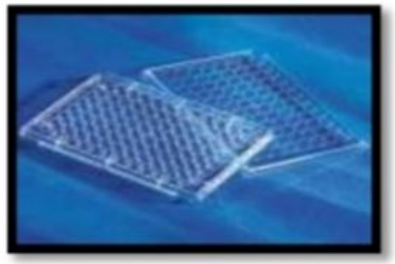
• Microwell-based EIAs for detection of antigens of several nonculturable gastrointestinal viral pathogens (rotavirus and adenovirus) are available.



Printer

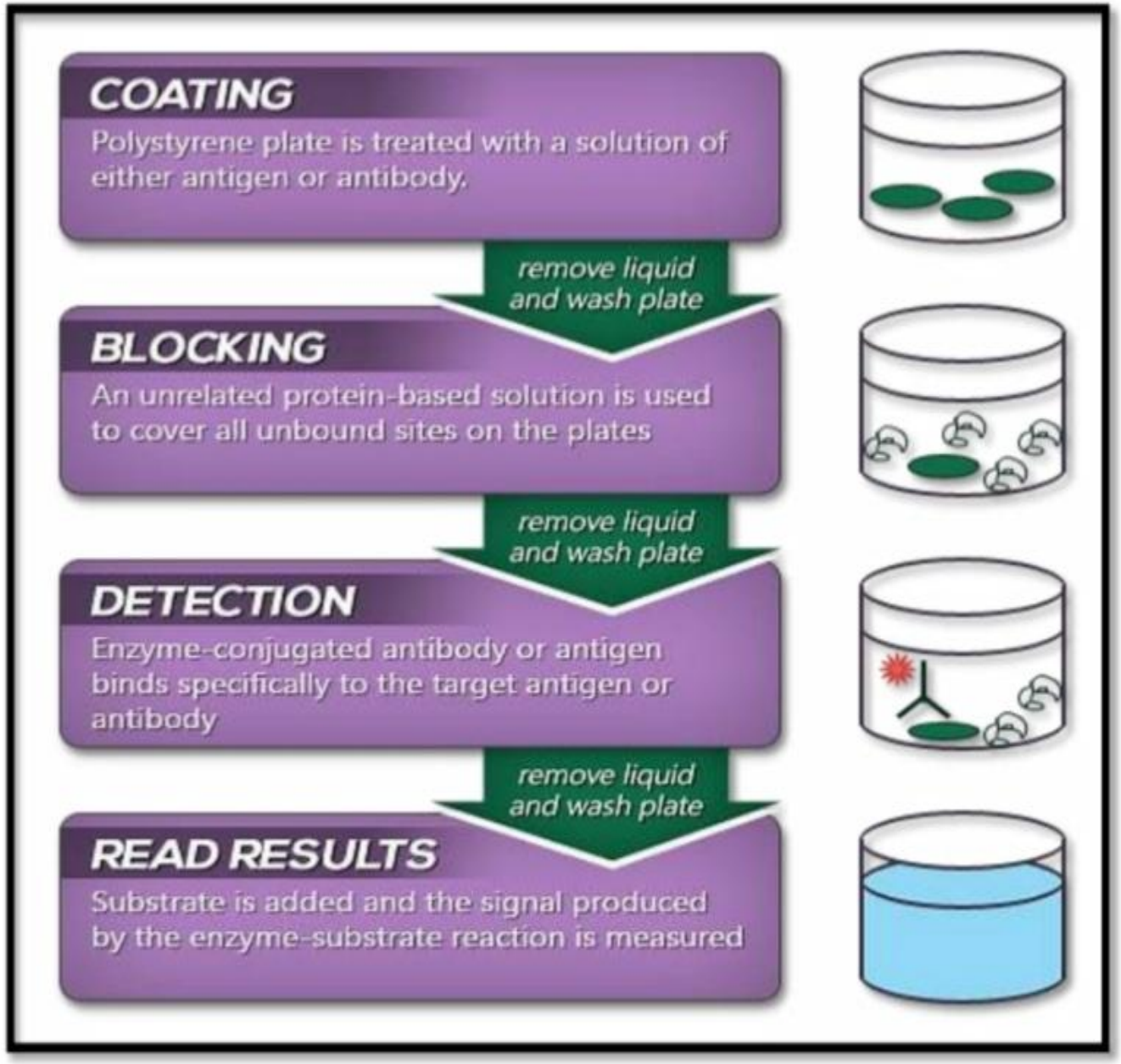
ELISA Reader

Micropipette

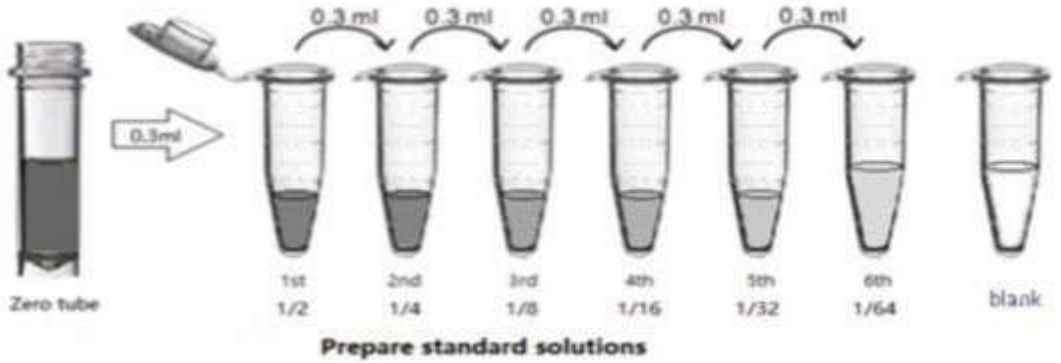


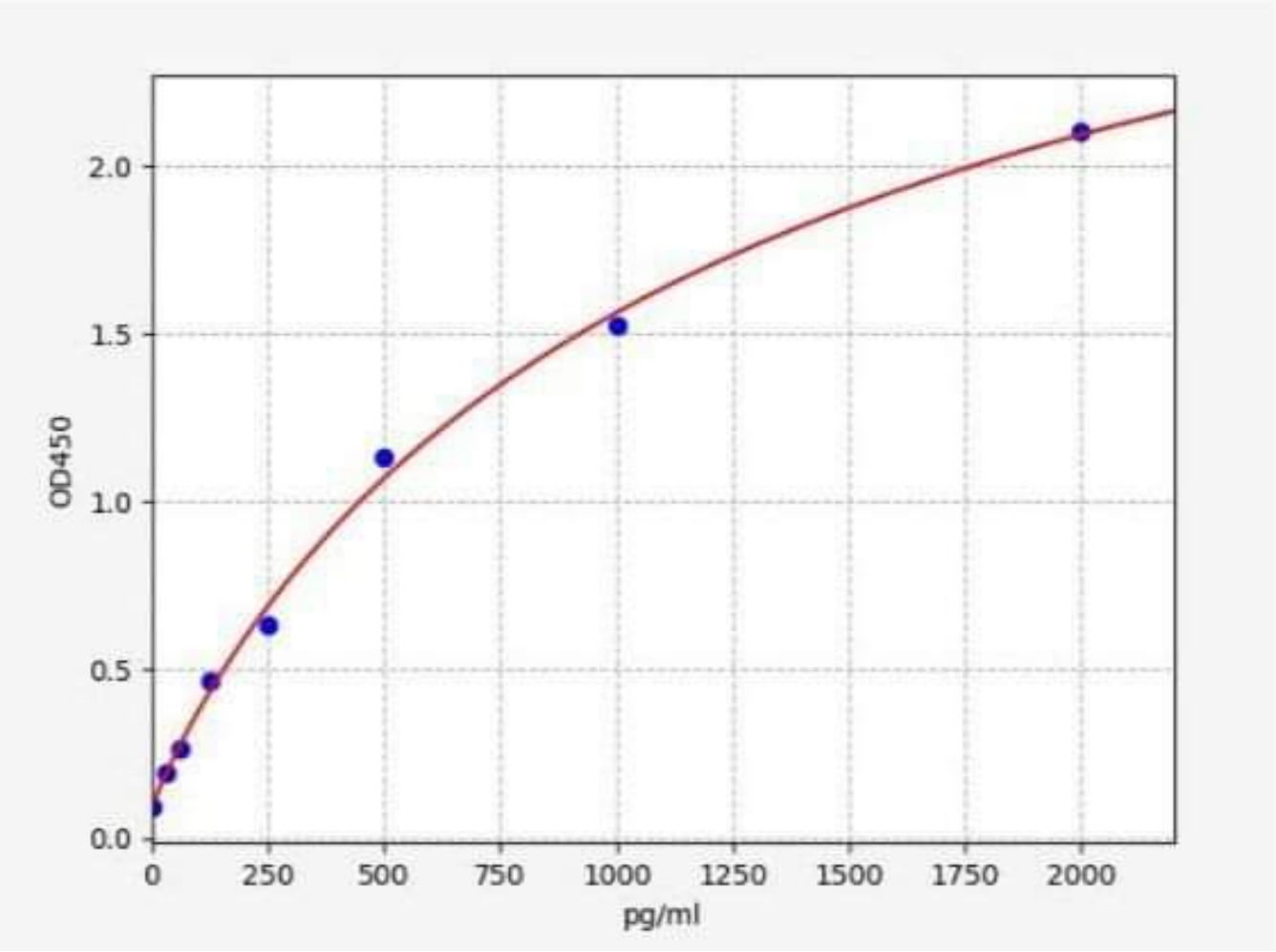
Microtiter plate


ELISA Kit




Steps of ELISA


0.3 ml
0.3 ml
0.3 ml
0.3 ml
0.3 ml
0.3ml
Zero tube
1st
1/2
2nd
1/4
3rd
1/8
4th
1/16
5th
1/32
6th
1/64
blank
Prepare standard solutions


OD450
2.0
1.5
1.0
0.5
0.0
0
250
500
750
1000
1250
1500
1750
2000
pg/ml

# Any questions?