



Medical laboratory techniques

Title of the lab. 5 :

Preservation and Titration of Virus

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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



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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.

Virus Quantitation

Chemical/Physical Methods of Virus Quantitation

Chemical/physical methods of virus quantitation measure the amount (or relative amount) of a viral protein, genome, or enzyme, in a sample. Types of chemical/physical methods include:

- 1. Direct visualization of virions by EM.**
- 2. Hemagglutination (HA) assay.**
- 3. Serological assays** (based on antigen–antibody interactions,). Examples of serologic assays include enzyme-linked immunosorbent assays (ELISA), fluorescent-tagged antibody assays, and precipitation assays.
- 4. Genome quantification by PCR.**



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IMMUNOLOGICAL TECHNIQUES

FIRST :- 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 inexpensive and does not require either sophisticated 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.



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