

# Viral Diagnosis

- It is a collection of all kinds of clinical and laboratory applications to be carried out for the diagnosis of virus infections.

Direct demonstration of the virus, its antigens or nucleic acid

**Viral (direct) diagnosis:** Diagnosis is the process by which the virus or viral structures (nucleic acid, protein sub-structures) are produced or detected.

**Indirect Diagnosis:** Diagnosis is made by serologic or biochemical detection of Ig that have been formed against viral infection after viral infection and also those that are released from degenerated or destroyed cells.

## Direct detection

Microscopy or staining

Detection of nucleic acid, antigens

Cell Culture cytopathic effect (CPE)

Embryonated eggs

Animals disease or death

**After Diseases with Respiratory System Symptoms:** Lunging lung tissue, mediastinal lymphatic lobe, tracheal scar, effusion sample.

**After Diseases with Digestive System Symptoms:** Lezyolu oral mucosa, esophagus, stomach and intestinal mucosa scrapings, tissue fragments, stomach and intestinal contents, mesenterical lymph ovaries, peyer plaques and asesites.

**Diseases with CNS indications:** CSF, MSS tissue.

**In Multisystemic Diseases:** Blood, lymphatic tissue (spleen, liver, lymph ovules), pericardium, effusion and asites liquids and all lesioned tissues

Samples can be sent to laboratory.

# Samples can be taken for Indirect Diagnosis

Indirect diagnosis is based mainly on blood serum samples taken from living or non-living animals.

Serological diagnostic procedures can be widely applied to blood serum as well as milk and CSF samples.

At least two fold antibody titer increase ( $B \leq 2 A$ ) in two blood samples (I and II) taken 15 days apart is indicative of an acute infection.

# Transferring samples

Samples should be sent to the laboratory in the cold chain.

The critical point is;

Cellular integrity should be maintained in order to avoid virus inactivation in samples for direct diagnostic purposes.

Transport liquids are used for this purpose;

- PBS (Phosphate buffered saline)
- PBS with 50% glycerin
- Serum Physiological (0.9% NaCl)

FORMOL - SHOULD NOT BE USED.

## RIGHT LABORATORY SELECTION

Examples should be sent to laboratories capable of studying the cause of the suspected disease;

- Republic of Turkey Ministry of Agriculture and Welfare
- Central Research Institute Laboratories (Rabies and many other viral infections)
- Footh and Mouth Disease Institute (foot and mouth disease)
- University Laboratories
- Special laboratories



# Virus Inoculation and Specimen Processing

**Specimen:** Any sample taken from suspected individuals (living or dead) from a virus infection.

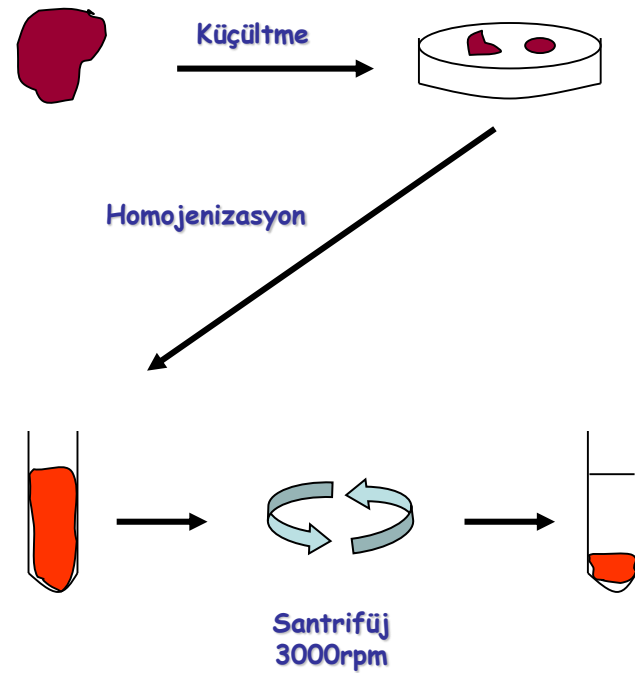
**Inoculum:** The inoculum is a specimen which is processed for inoculation into cell cultures and all kinds of microorganisms are removed from microorganism.

Removal of the treated sample from non-virus pathogens occurs at the following steps;

- Concentrated antibiotic depletion (Pen, Strep, Kana)
- Filtration (0.45-0.6 nm porosity cellulose acetate filters)
- High-speed centrifugation (15000-20000 rpm)
- Treatment with oil solvents

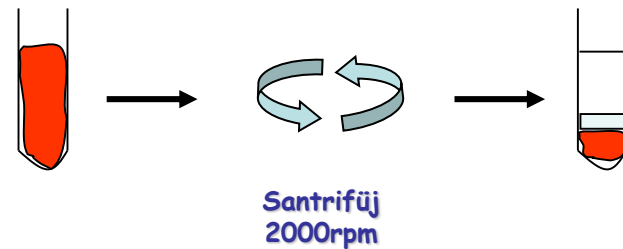
# Organ Material

- Organ surface disinfection
- Reduction
- Dilution and homogenization in 1/10 PBS
- Centrifuge at 3000 rpm
- The supernatant is removed, sterilized by antibiotic addition
- Inoculation to agar plate and control
- Storage at -80oC or inoculation to cell cultures



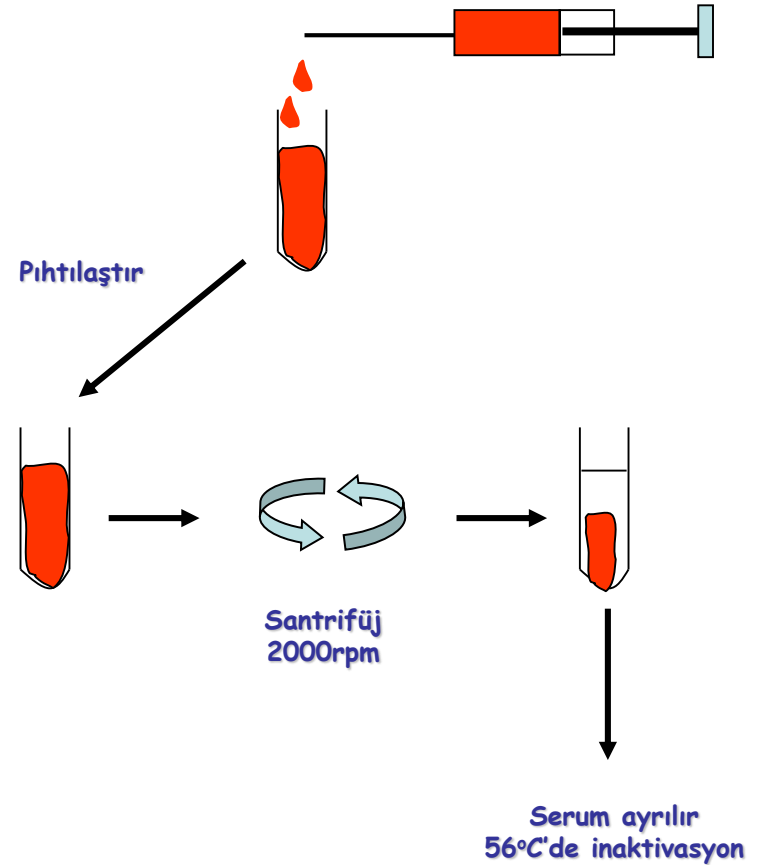
# Blood (For Virus Isolation)

- Blood is taken from anticoagulant
- And centrifuged at 2000 rpm for 10 minutes at 4 ° C.
- Leukocyte layer capillaries formed in the middle are collected by pipette and resuspended in 2-3 mL of PBS.
- It is centrifuged under the same conditions.
- Leukocytes recapture. This process is repeated 2-3 times.
- Leukocytes from the last wash are either immediately inoculated into the cell or frozen by placing the cryopreservative.



# Blood (for Serological Diagnosis)

- Without any substance glass or blood containing kaolin is taken.
- In the course of clotting, the clot is separated from the tube wall by a wire.
- And centrifuged at 2000 rpm for 10 minutes at 4 ° C.
- The obtained serum is picked up in a clean scarf.
- It is inactivated at 56 ° C for 30 minutes before use.
- If not to be used in tests
- Frozen at -20 ° C.



# Feces

- Dilute and homogenize 1/10 in antibiotic PBS.
- Centrifuge at 3000 rpm
- The supernatant is removed, sterilized by antibiotic addition
- inoculation to agar plate and control
- Storage at -80oC or inoculation to cell cultures

# Swap

- Samples from the laboratory are vortexed in 2-3 ml of antibiotic PBS.
- Cotton is squeezed in the tube wall and cotton is discarded.
- The liquid is centrifuged at 3000 rpm for 10 minutes.
- Antibiotics are added and sterility checked.
- Cell cultures are inoculated or frozen.

