

EXERCISE - 2

- **Preparation of viral inoculum from tissue sample for cultivation of virus in lab animal, embryonated egg and tissue culture**

REQUIREMENT –

- Biosafety cabinet
- tissue sample in sterile sample collection vial,
- kidney tray
- 1 wide petridish & 2 small petridish
- two scissors straight and curved,
- forceps,
- weighing machine,
- aluminium foil,
- sterile pestle-mortar,
- sterile sand,
- TPB-Tryptose phosphate broth,
- Micropipette 1000 μ l
- Microtips 1000 μ l
- funnel,
- sterile centrifuge tube,
- centrifuge machine,
- penicillin,
- streptomycin
- Apron,
- Spirit,
- Cotton swabs

PROCEDURE -

Collect samples i.e. infected tissue or exudate from the animal with the help of sterile instrument and place it in sterile container



Weight 1gm of tissue, cut into small pieces and place in sterile mortar



Sprinkle small amount of sand over the tissue



Grind tissue to a paste



Suspend it in 9ml of TPB (Tryptose phosphate broth) to make 10% suspension



Transfer suspension in centrifuge tube and centrifuge at 3000rpm for 3-5 minutes



Collect supernatant in sterile tube



Add antibiotics (Penicillin 100IU/ml & Streptomycin 100µg/ml)



Incubate for 15-30 minutes at room temperature



Inject/inoculate suspension in animals/Chicken embryos