Post Graduate (P.G.), Monsoon semester

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# **VMC 609:** TECHNIQUES IN MICROBIOLOGY AND IMMUNOLOGY

## **TOPIC:**

*" Methods of preservation of virus - cryopreservation"*

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# PRESERVATION OF ANIMAL VIRUSES

- Including freeze
- Freeze-drying
- Dehydration by physical drying
- Chemicals
- In vitro culture
- Freeze-drying is the most widely and reliable method

# CRYOPRESERVATION

- Widely used for long-term preservation of animal and human viruses
- Virus infectivity is retained well at temperatures below 60°c.
- Viruses are frozen rapidly and this is accomplished by storing only small volumes (0.1 to 0.5 ml) of virus suspension.

- Long term storage at - 20°C of acetone or paraformaldehyd fixed virus infected cells on glass coverslips is a very convenient method of retaining specific virus antigens for serodiagnostic purposes.

- Virus preparations to be preserved from tissue culture monolayers, cell suspensions or allantoic fluid from infected fertile hen eggs, should be clarified by centrifugation at about 3000 rpm for 10 minutes at 4°C.

# PROCEDURE

- The clarified preparation should then be dispensed and frozen immediately.
- Label the cryotubes in which it will be preserved
- Place the flask containing the clarified virus suspension, either as tissue culture supernatant medium or as cell lysate (in tissue culture medium), in an ice bath for a few minutes.

- Dispense small volumes (from 0.1 ml to 1ml) of the clarified medium aseptically into the cryotubes using a sterile pipette and ensuring that the cap of each cryotube is screwed down firmly.

- Transfer the cryotubes containing the dispensed virus directly to the 70°C freezer.

- For experimentation it should be placed in a water bath at 37°C immediately and removed as soon as it has thawed. Use the virus as soon as possible after thawing keeping it at 4°C until used.

# INTRODUCTION

- Cryopreservation: Storage of live material at temperatures so low, that all biological processes are suspended (-196°C) and material does not decompose.
    - Cryopreservation allows virtually indefinite storage of biological material without deterioration over a time scale of at least several thousands of years.
    - Techniques are available for the preservation of microorganisms, isolated tissue cells, small multicellular organisms, and even more complex organisms such as embryos.

# CRYOPRESERVATION IN LIQUID NITROGEN

- Cut a length of tubing sufficient to extend 2 cm beyond each end of the Cryotube.
- Insert the correctly labelled cryotube containing the virus in the centre of the cut length of Cryo tubing.
- Heat the tubing gently using the flame from a Bunsen burner or heat gun

- The heat will shrink the tubing around the Cryotube.

- Reheat the ends of the tubing and squeeze or crimp the ends with a large pair of forceps (or equivalent) to provide a seal.

- The ends of the Cryotubing should be melted to ensure an absolute seal.

# THANK YOU

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