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Popular Article

Long-Term Preservation of Animal Viruses: An Overview

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Introduction

Preservation of animal viruses for long term storage possess a significant challenge due to the delicate nature of the viruses. The traditional methods include cooling or freezing that can lead to ice crystal formation, membrane damage, osmotic shock and rendering the viruses non-infectious. Infectivity can also be compromised by degradative enzymes, detergents that solubilize lipid-containing envelopes. However, advanced virus storage techniques involve freeze-drying and ultra-freezing techniques. It has been proven that viruses can be preserved as infectious agents by being stored at low or ultra-low temperatures, or in the absence of water. This approach helps to mitigate the risks associated with ice crystal formation and other damaging effects. Additionally, the incorporation of supplements like serum is presumed to stabilize environmental conditions and impede degradative processes. The efficacy of these preservation methods varies, as different viruses exhibit unique properties that influence the choice of specific procedures for maintaining virus stocks. Overall, the balance between temperature control, moisture elimination, and the addition of stabilizing agents plays a crucial role in successful long-term storage of animal viruses.

General rules applied for the preservation of most of the viruses

There are general guidelines that must be applied for the maintenance of viruses, assuring their stability throughout time. Freeze-dried virus preparations can be preserved for decades when stored at 4°C in the dark, providing a reliable method for long-term conservation. Liquid nitrogen

has been proved for retaining virus infectivity for extended periods, making it a preferred choice for preservation. Preserving small volumes of virus suspension is considered good practice that aids in maintaining the virus's integrity. Furthermore, for the long-term storage, high titer virus preparations are preferred. When dry ice is used for preservation, it is crucial to employ totally sealed containers to prevent potential contamination or loss of viability. These guidelines contribute to the establishment of effective and reliable practices for the long-term preservation of viruses.

Cryopreservation

Cryopreservation is a versatile approach for storing viruses, particularly when the retention of virus infectivity is not crucial. When the sample serves as an antigen in ELISA tests, it can be stored at -20°C for many years without a significant loss of antigenic activity, even though infectivity may be reduced. For those cases where preserving infectivity is important, temperatures below -60°C are found to be particularly effective. The availability of freezers capable of maintaining ultra-low temperatures has facilitated this practice. Among the ultra-low temperature options, -70°C is commonly favoured for its efficacy in preserving virus infectivity. For optimal cryopreservation, viruses should be frozen rapidly, and should be stored in small volumes (0.1–0.5 mL) of virus suspension.

Cryopreservation at 4°C and –20°C

Cryopreservation techniques at 4°C and -20°C offer effective means for the storage of bacteriophages. The majority of bacteriophages exhibit robust stability and can be maintained at 4°C for several years. Although the infectivity of these bacteriophages may experience a gradual decline over time, the stock can be easily rejuvenated after one or two years by culturing the phage in the suitable bacterial host. Cryopreservation techniques can also be used to successfully maintain even more complex viruses, such as baculoviruses or pox viruses. These practices contribute to the sustained availability of viral stocks, providing researchers with valuable resources for various experimental and research endeavors.

Snap freezing

Also known as flash freezing, is a rapid cooling technique employed to lower temperatures below -70°C, typically utilizing dry ice or liquid nitrogen. The process involves the use of a Cool rack, designed for fast heat transfer, ensuring the specimen is rapidly cooled. This method is favoured for preserving the integrity of specimens, maintaining their structural and biochemical

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characteristics.

Cryopreservation at -70°C

Cryopreservation at -70°C is a highly effective method for maintaining the infectivity of viruses. The use of freezers designed to maintain ultra-low temperatures is crucial for the success of this preservation technique, with -70°C (or more recently, -80°C) being the preferred temperature. To ensure the integrity of stored samples, it is advisable to use freezers equipped with alarms that warn of any temperature rise exceeding 5°C, preventing potential damage due to temperature fluctuations.

Procedures

Effective sample storage at -70°C involves meticulous steps. Cryotubes are labeled for identification, and a clarified virus suspension is dispensed into them in small volumes (0.1 to 1 mL). The cryotubes are organized in storage racks within a -70°C freezer. Thawing is done promptly in a 37°C water bath, and the thawed virus should be used immediately or kept at 4°C for stability. This method ensures optimal viability and integrity.

Cryopreservation in liquid nitrogen involves the use of small volume cryotubes for storage. To ensure the integrity of the storage process, cryotubes must be sealed in specialized tubing, such as Nunc Cryoflex. This precaution is crucial to prevent the risk of cross-contamination of viruses and to minimize the exposure of the operator to virus-containing aerosols when cryotubes are removed from the nitrogen. The storage tanks must undergo regular checks and be replenished with liquid nitrogen as needed.

Storage in liquid nitrogen requires sealing cryotubes in specialized tubing, such as Nunc Cryoflex, to prevent cross-contamination and reduce aerosol exposure. Regular checks and replenishment of liquid nitrogen in storage tanks are essential. The procedure involves inserting a labeled cryotube into tubing followed by heating the tubing and freezing in liquid nitrogen, and storing in a tank for long-term preservation.

Freeze drying viruses for long term preservation

Freeze-drying is also called as sublimation drying. It is a method employed for the long-term preservation of viruses. This process involves drying a product while it is in a frozen state without passing through the liquid phase and making it the most satisfactory technique for maintaining the viability of viruses over extended periods.

Vacuum Sealing for Small Sample Processing

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For small sample sizes, a simple and efficient method involves a single vacuum stage using 5 ml or 2 ml glass ampules. These ampules, designed with a slender neck and a snap-off point, facilitate the preservation process. Introducing a small restriction in the neck simplifies the vacuum sealing process, ensuring a robust seal for prolonged sample preservation.

Disadvantages of Freeze-Drying

While freeze-drying offers advantages, notable disadvantages include the high capital cost of specialized equipment, making it economically less viable for some applications. Additionally, the process incurs high energy costs due to the maintenance of extremely low temperatures. In summary, drawbacks include high capital investment, elevated energy costs, and prolonged processing times.

Conclusion

Preserving animal viruses for long-term storage demands a meticulous approach, considering factors like temperature control, moisture elimination, and stabilizing agents. Advanced techniques such as freeze-drying and ultra-freezing, along with general guidelines for low temperatures and small volumes, offer effective preservation solutions. Cryopreservation techniques cater to various viruses, while snap freezing maintains structural integrity. Despite advantages, freeze-drying comes with drawbacks like high costs and prolonged processing times. Researchers must navigate these considerations to employ tailored preservation strategies, ensuring the availability of valuable resources for prolonged virus storage.

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