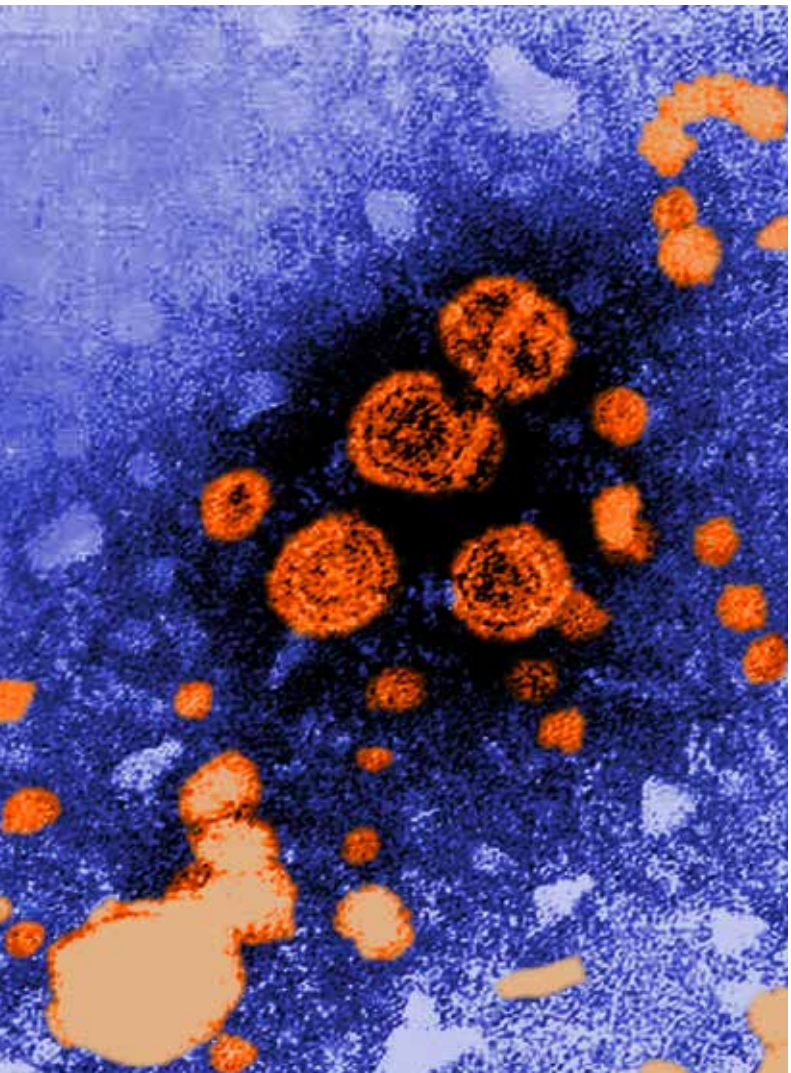
ATCC® VIROLOGY GUIDE

Tips and techniques for propagating virus in tissue culture and embryonated chicken eggs



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This guide contains general technical information for viral growth, propagation, preservation, and application. Additional information on viral culturing can be requested from ATCC Technical Services at www.atcc.org or can be found in *A Manual of Basic Virological Techniques*[1].

Getting Started with an ATCC Viral Strain

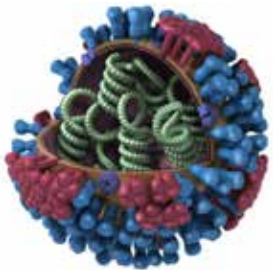
ATCC viral strains are predominantly shipped either frozen on dry ice, in plastic cryopreservation vials, or as lyophilized materials within glass ampoules or serum vials. Upon receipt of frozen material, immediately thaw and transfer the viral agent to an appropriate propagation host. Alternatively, the frozen material can be stored between -70°C and -80°C for short periods (1 to 5 days); however, viability of some materials may decline at temperatures above -120°C. Upon receipt of freeze-dried strains, reconstitute cultures with sterile, double-distilled water and add the rehydrated material to an appropriate propagation host. If this is not possible, store the vials in liquid nitrogen vapor phase (below -120°C).

Product Sheet

ATCC viral strains are shipped with a product sheet that contains information on the production host and recommendations for infection. The product sheet and additional information can be found on the ATCC website or can be requested from the ATCC Technical Service Department.

Viral Taxonomy

Viruses are placed into taxonomic groups based on characteristics including morphology, genome type, and host organism. Viral agents can significantly vary in size, often ranging between 20 and 300 nanometers in diameter. They also vary in structure, including **helical**, **icosahedral**, **prolate**, **enveloped**, and **complex** morphologies.



Influenza ultra-structure courtesy of Jordan Douglas, CDC

In addition to unique morphological structures, viruses also vary in genomic structure. Unlike other microorganisms that have double stranded DNA as genomic material, viral genomes can be composed of double-stranded DNA, single-stranded DNA, double-stranded RNA, or single-stranded RNA. Single-stranded RNA viruses can be further described as **positive sense**, **negative sense**, or **ambisense**. For more detailed information on the various morphological and genomic types, please refer to the glossary.

Changes in taxonomy or further analysis of viral strains may lead to a change in nomenclature. Taxonomic nomenclature as well as the common name can be found on the product sheet. Further information on viral nomenclature is available online at http://ictvonline.org/.

Viral Replication

Viruses are pathogenic intracellular organisms requiring living cells in order to multiply. The virus life-cycle can be divided into three major steps: attachment, assembly, and release. Generally, infection is established when the virus binds to a specific cellular receptor and enters the host cell. Upon cellular entry, host proteins are recruited to assist with viral replication. Once viral structural proteins are generated, new viruses assemble within the cell. Depending on the nature of the viral agent, the replication and assembly process can vary in cellular location and process. Following viral assembly, new infectious particles either remain cell-associated or exit the cell via lysis or virus shedding.

Preparation of Propagation Host and Reagents

In advance, prepare the appropriate propagation host and associated reagents necessary for viral propagation. Information for the preparation of these products is available on the provided product sheet.

Opening Glass Ampoules Containing Frozen Material

Overview

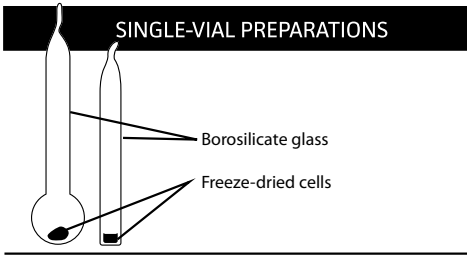
All cultures should be considered potentially hazardous and should be opened by individuals trained in microbiological techniques. Work should only be carried out in facilities with containment requirements appropriate for the biosafety level of the cultures. ATCC recommends that the handling or opening of glass ampoules be performed in a biological safety cabinet. If this is not possible, wear protective clothing, gloves, a face shield or safety goggles, and hold the vial away from your body. Ensure that all empty vials are sterilized before disposal.

1. Disinfect the outside of the ampoule with freshly prepared 70% ethanol or dip it into a beaker of freshly prepared 70% ethanol.
2. To recover the material from the glass ampoule, score the neck of the ampoule with a sterile, small file.
3. Wrap the ampoule within several folds of a sterile towel or gauze to dry residual ethanol.
4. Working in a biological safety cabinet, hold the vial upright and snap open the vial. Ensure that your gauze does not become too wet with ethanol, or ethanol could be sucked into the culture when the vacuum is broken. Propagate the virus immediately.

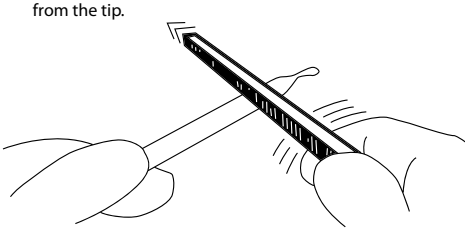
Initiating Frozen Cultures

Tissue Culture-Adapted Strains

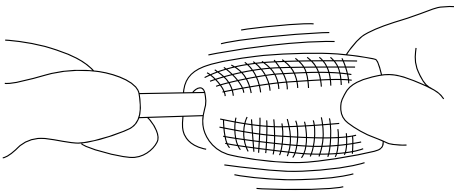
1. In advance, prepare the appropriate cell growth medium for growing the host cell line. Additionally, prepare the appropriate virus growth medium for virus propagation as noted on the product sheet. Viral growth medium is usually supplemented with a lower percentage of serum than cell growth medium, often ranging between 2-10% depending on the virus (See NOTE 1). Ensure that both the cell



1 These preparations may be enclosed in a thin skin of cellulose; this skin must be removed (either with a sharp blade or by soaking in water for a few minutes). Score the ampule once briskly with a sharp file about one inch from the tip.



2 Disinfect the ampule with alcohol-dampened gauze



3 Wrap gauze around the ampule, and break at the scored ar Care should be taken not to have the gauze too wet, or alcohol could be sucked into the culture when the vacuum broken. Rehydrate material at once.