LONG-TERM AND SHORT-TERM STABILITY OF VIRUSES DEPEND ON STORAGE TEMPERATURE AND PRESERVATION METHOD

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Abstract

The ability to store viruses for long periods of time with minimal loss of viability is critical. Even more than most organisms, viruses are fragile and degrade rapidly unless stored at low temperatures. The ATCC® and BEI Resources collections offer a unique opportunity to study the stability of various viruses under different conditions. The ATCC® Virus Facility, which celebrates 50 years of operation in 2008, contains more than 2,500 different holdings spanning decades of storage time using many different storage methods. A retrospective study was performed to evaluate the short- and long-term viability of various holdings of influenza virus in the ATCC® collection. Using virus isolates that had been banked and stored as long ago as 1960-61, a variety of storage methods were employed at ATCC® and BEI Resources for the storage of viral preparations. Storage in liquid nitrogen in the vapor phase in the ATCC® facility offers the benefits of simple, safe storage and excellent recovery.

Materials and Methods

In this study, the viability of Influenza and Parainfluenza viruses (Table 1) grown in embryonated chicken eggs, stored as wet frozen preparations from -180°C to -70°C and freeze-dried preparations stored at -70°C to 37°C were evaluated. All viruses were obtained directly from the ATCC® Animal Virus Collection, stored in the main Repository or were part of an ongoing long-term viability study started in 1996 at the ATCC®. All viruses were grown in 9-11 day old specific pathogen free (SPF) embryonated chicken eggs and incubated for 32-33 h at 33°C.

To test the stability of an influenza or parainfluenza virus, each virus is titered by making 10-fold serial dilutions in DPBS and inoculating these into SPF embryonated chicken eggs. Each dilution of virus is inoculated into three SPF embryonated chicken eggs by centrifugation of the virus infected allantoic fluid. The virus is harvested by collecting virus infected allantoic fluid from appropriately labeled tubes and testing it by hemagglutination. Hemagglutination is obtained when the hemagglutination titer of the virus exceeds 1,024. If hemagglutination is obtained, samples of the virus are stored as wet frozen preparations at -70°C in 50% DMSO. If the virus is not stable as the Influenza A virus tested in Figure 1, but this isolate has maintained its original viability for 10 years at -70°C and 20°C, which results were unable to be repeated. The purpose of archival storage of biological materials is to offer historical resources for researchers to use to broaden their research. Storing viral material at consistent temperatures over long periods of time to maintain viability is of little or no value. The current study has shown that archived Influenza A and Parainfluenza type 1 viruses show little or no loss of viability after 10 years at -70°C and 20°C. Results from viability testing covering a 10 year span showed that one isolate of each of Influenza A, Influenza B and Parainfluenza type 1 viruses can maintain viability for 10 years at 20°C and temperatures greater than 20°C. The primary goal of this study was to test the hypothesis that archival storage at colder temperatures show lower rates of decay. This hypothesis was tested in this study.

Conclusions

An archival preparation of virus is usually thought to be a viable sample of virus suspension stored at -70°C for 10 years. In this study we have shown that for the viruses examined here, archival virus stocks do maintain similar viability as when initially prepared. The current study further shows that there are other options available for long-term archival storage that is not primary goal and demonstrates the ability to store virus samples at temperatures greater than -70°C and still have viable virus. Storing viral material at consistent temperatures over long periods of time to maintain viability is of little or no value. The current study has shown that archived Influenza A and Parainfluenza type 1 viruses show little or no loss of viability after 10 years at -70°C and 20°C. Results from viability testing covering a 10 year span showed that one isolate of each of Influenza A, Influenza B and Parainfluenza type 1 viruses can maintain viability for 10 years at 20°C and temperatures greater than 20°C. The primary goal of this study was to test the hypothesis that archival storage at colder temperatures show lower rates of decay. This hypothesis was tested in this study.

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References