

# Stability of Viral Pathogens in the Laboratory Environment

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## Abstract

*Knowledge of the stability of pathogens in the environment is part of a comprehensive biological risk assessment. The inherent nature of laboratory equipment and laboratory procedures to create aerosolized droplets of infectious agents, with subsequent deposition of these particles, provides opportunities to contaminate fomites such as laboratory equipment and personal items. Vivaria create an increased level of virus amplification and fomite contamination as the dried waste of virus-infected research animals can become aerosolized, or transmitted through direct contact. This paper provides a review of studies in which several species of virus have been allowed to dry on porous, or nonporous substrates, and analyzed at timed intervals to determine the ranges over which these viruses remain stable at room temperature as measured by their viability to infect cell cultures or research animals. The research shows that some viruses retain viability for up to a month or longer in the laboratory environment. This review will aid investigators and biosafety professionals in both risk assessment as well as decontamination efforts.*

## Keywords

Virology, viral stability, biosafety, environmental stability, biosecurity, risk assessment, biocontainment, disinfectant, decontamination

## Introduction

Biological risk assessment, as defined in the *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* (5th Edition), is a process used to identify the following: the hazardous characteristics of a known infectious, or potentially infectious agent or material; the activities that can result in a person's exposure to an agent; the likelihood that such exposure will cause a laboratory-associated infection; and the probable consequences of such an infection. Both the *BMBL* and the World Health Organization's (WHO) *Laboratory Biosafety Manual* (3rd Edition) include pathogen stability in the environment as an essential component in conducting risk assessments. Although much is known about the ability of bacterial pathogens to survive for great lengths of time in various environments in dormant states, several studies on viral pathogens have shown a remarkable ability of these agents to remain viable in the laboratory and vivarium environment.

Previous reports have focused on the infectivity of aerosolized virus (Peters et al., 1996) and viral survival

under general environmental conditions (Pirtle & Beran, 1991). In addition, reviews concerning a select few human pathogenic viruses under laboratory conditions have been considered (Mahl & Sadler, 1975; Sattar & Springthorpe, 1996; Kramer et al., 2006). The objective of this review is to examine the stability of viruses on surfaces with respect to conditions typically found within the context of the laboratory environment. Virus studies pertinent to the field of laboratory biosafety have been tabulated if they were conducted at 25°C (plus or minus 5°C) and a relative humidity of 50% (plus or minus 10%). Typically, these viruses were deposited in buffer onto porous (cloth or gauze) or non-porous (metal or glass) objects and allowed to dry at room temperature at 50% relative humidity. At measured time points from this initial deposition, samples were re-suspended in buffer, and either inoculated onto cells in Petri dishes, or in some cases, directly into animals to determine the viability of the virus to cause cytopathic effect, or infection. The reader is invited to review primary literature on a case-by-case basis to determine the specific conditions for each virus, suspension buffer, and cell type used when applying data toward the research group's own risk assessment.

Viruses included in this review are bloodborne (such as HIV and Hepatitis B), possess the potential to be used as vectors (such as HIV, adenovirus, and vaccinia), viruses transmitted readily via the aerosol route (such as influenza virus and coronavirus), and/or possess special considerations with regard to vivaria.

## Bloodborne Pathogens

In 1991, the U.S. Department of Labor Occupational Safety and Health Administration (OSHA) set forth Regulations (Standards-29 Code of Federal Regulations), Bloodborne Pathogens Standard 1910.1030 to protect workers at risk of coming into contact with potentially infectious pathogens found in blood and body fluids. These pathogens include, but are not limited to, viruses including hepatitis B virus, hepatitis C, and human immunodeficiency virus (HIV) (Table 1). Viruses present in blood and body fluids are surrounded by a high organic load carrying a high potential to remain viable for long durations.

Lentiviral vectors include: human vectors based upon HIV, and vectors from non-human sources based upon feline immunodeficiency virus, simian immunodeficiency virus, or equine infectious anemia virus, as well as hybrid HIV/SIV (SHIV) vectors. According to the Recombinant DNA Advisory Committee Guidance Document, either BL2 containment, or enhanced BL2 containment, is ap-

appropriate in the laboratory setting for research involving the use of advanced lentivirus vector systems possessing multiple safety features, and that segregate vector and packaging functions onto four or more plasmids (RAC, 2006). Researchers will continue to use HIV and retroviral vectors as these viruses allow for the incorporation of retroviral nucleic acid into the cellular genome.

### Adenovirus

The family Adenoviridae includes double-stranded DNA viruses lacking a lipid envelope. Adenoviral vectors are widely used in *in vitro* and *in vivo* gene transfer studies in both animal models and clinical trials (Chuah et al., 2003). Adenoviruses should not be confused with adeno-associated viruses, a virus in the family Parvoviridae, since both of these viruses are used in gene transfer studies.

Adenoviruses and their cognate vectors differ in that the parental virus can cause a productive infection associated with viral replication, whereas adenoviral vectors are replication impaired (Chuah et al., 2003). Adenovirus remains a cause of respiratory infections in children and is readily transmitted in daycare centers. Given the prolonged viability from one week to 12 weeks (Table 2), researchers using viruses in this family should consider employing disinfectants suitable for these membrane-lacking viruses.

### Influenza Virus

Members of the family Orthomyxoviridae are enveloped viruses containing genomic RNA in eight segments.

Although seasonal influenza causes significant morbidity and mortality, reconstructed 1918 influenza and avian influenza pose special cases with regard to biocontainment procedures. Of special note is the viability for up to six days on steel and cotton fabric for low pathogenicity avian influenza (Tiwari et al., 2006), an important consideration for livestock, poultry, and human pathogen research, considering that different influenza strains have the ability to readily cross from species to species (Table 3).

The virus' RNA genome is highly susceptible to mutation by virtue of being constructed by the viral RNA polymerase, in addition to the unique constellation of eight RNA strands that are inherited from different parental strains. The wide range of viability reported for environmental influenza strains may be attributed to the disparate biological properties expressed by the unique combination of genes found in each strain. A recent study of four different strains on bank notes showed a viability ranging from two hours to five days (Thomas et al., 2007). As such, each strain of influenza being studied must undergo a thorough risk assessment prior to research.

### Vaccinia Virus

The family Poxviridae contains a variety of vector candidates, from a replicative virus exhibiting some virulence in animal models (such as wild-type vaccinia virus) to a non-replicative virus with no detectable virulence even when tested in severely immunocompromised animals (such as highly attenuated vaccinia strains or canarypox virus) (Vanderplaccen & Pastoret, 2003). As such,

**Table 1**

Environmental stability studies on viral bloodborne pathogens. Hepatitis B virus is a lipid enveloped member of family Hepadnaviridae and has a partially double-stranded DNA genome. HIV is a lipid-enveloped member of the family Retroviridae and has two genomic RNA strands.

Virus	Survival Time	Substrate	Authors
Human T-cell Lymphotropic virus (HTLV-III-TM)	>3 days	culture plates	Resnick <i>et al.</i> , 1986
HIV-1 HTLV-III B strain)	>34 days	glass slide	Tjotta <i>et al.</i> , 1991
Hepatitis B	>7 days	silanysed screwcap tubes	Bond <i>et al.</i> , 1981
HIV-1 (RF strain)	4 - 8 wks	glass cover slide	van Bueren <i>et al.</i> , 1994

**Table 2**

Environmental stability studies on Adenovirus.

Virus	Survival Time	Substrate	Authors
Adenovirus 19	8 - 10 days	paper, cloth	Nauheim <i>et al.</i> , 1990
Adenovirus 3	10 days	paper gum (dextrin)	Selwyn, 1965
Human Adenovirus type 3	>9 days	polystyrene Petri dish	Rabenau <i>et al.</i> , 2005
Adenovirus 19	35 days	plastic	Nauheim <i>et al.</i> , 1990
Adenovirus-2	8 - 12 wks	glass slides	Mahl <i>et al.</i> , 1975

there have been several incidents of Laboratory-Associated Infections (LAIs) reported within the last ten years (Rupprecht et al., 2001; Mempel et al., 2003; Mous-satché et al., 2003; Lewis et al., 2006).

The one study that was found in the literature search indicates the virus has a robust survival time despite being an enveloped virus (Table 4) (Mahl et al., 1975). Similar to HIV above, a rich organic load sustains viability of the smallpox virus. MacCallum and McDonald showed that the smallpox virus is stable in crusts of patients for two to four months depending on the humidity of the stored material (Essbauer et al., 2007). Due to the highly infectious nature of this virus, the Advisory Committee on Immunization Practices guidelines recommends the use of vaccinia vaccine to protect three classes of people susceptible to infection: 1) laboratory workers working with nonvariola Orthopoxviruses (e.g., vaccinia and monkey-pox); 2) persons working in animal care areas where studies with Orthopoxviruses are being conducted; and 3) health-care workers involved in clinical trials using recombinant vaccinia virus vaccines (ACIP, 2001).

### Zoonotic Viruses

Viruses used in animal research studies have the ability to be amplified within the laboratory animal to high concentrations. Viruses may be introduced into the animal through experimental protocol, or through unintentional infection from feral animals in the animal facility due to faulty pest management systems. In the former case, viruses may be inherently infectious to the laboratory animal, or attenuated to become infectious to the laboratory animal. In the latter case, unintended virus infections may disrupt experiments by either causing mor-

bidity of laboratory animals, or cease experiments altogether by causing mortality. An important consideration is that “attenuation” does not necessarily mean “less virulent.” Attenuation of virus in an animal model may likely increase virulence in humans. For these reasons, several different viruses seen in animal populations are provided (Table 5).

The potential for viruses infecting rodents (e.g., rat virus) to remain viable for up to seven weeks on bedding, and up to five weeks on plastic surfaces, indicates that fomites and equipment must be decontaminated, or disposed of properly in order to halt viral transmission in vivaria. The rat coronavirus was viable up to three days on plastic plates. Avian metapneumonovirus and low pathogenicity avian influenza display survival up to six days. Heightened awareness of biosecurity and biosafety measures as they pertain to the use of small mammals in vaccine trials, or biocontainment practices in the laboratory are therefore indicated.

### SARS Virus

The severe acute respiratory syndrome (SARS) coronavirus (CoV) appeared in China in 2002 and became a pandemic within months. SARS-CoV ultimately infected more than 8,000 people in late 2002 and 2003. Researchers scrambled to investigate this unknown virus and the genome was sequenced within one year of emergence.

Coronaviruses exhibit a moderate ability to retain viability on different substrates (Table 6). This family of viruses possesses a lipid envelope and a plus-sense single-strand RNA genome. The highly infectious nature of the disease, coupled with inappropriate laboratory standards and practices led to at least three instances of LAIs in

**Table 3**

Environmental stability studies on Influenza virus.

Virus	Survival Time	Substrate	Authors
Influenza A (PR8 strain)	>3 hours	stainless steel	Sattar <i>et al.</i> , 2006
Influenza B	48 hours	stainless steel	Bean <i>et al.</i> , 1982
Influenza A (H1N1)	72 hours	stainless steel	Bean <i>et al.</i> , 1982
Low Pathogenicity Avian Influenza	~6 days	steel	Tiwari <i>et al.</i> , 2006
Influenza A (PR8 strain)	> 3 days	cloth sheet	Edward <i>et al.</i> , 1941
Low Pathogenicity Avian Influenza	~6 days	cotton fabric	Tiwari <i>et al.</i> , 2006
Low Pathogenicity Avian Influenza	~6 days	plastic	Tiwari <i>et al.</i> , 2006
Influenza A (PR8 strain)	4 wks	glass slides	Edward <i>et al.</i> , 1941

**Table 4**

Environmental stability study on Vaccinia virus, a lipid-enveloped virus with a DNA genome.

Virus	Survival Time	Substrate	Authors
Vaccinia virus (Lederle strain)	4 - 5 wks	glass slides	Mahl <i>et al.</i> , 1975

**Table 5**

Environmental stability studies on zoonotic viruses.

Virus species	Survival time	Substrate	Lipid envelope	Genome Nucleic Acid Type	Family	Authors
Marburg virus (strain Popp)	4 - 5 days	wool	yes	ss(-)RNA	Filoviridae	Belanov <i>et al.</i> , 1996
Avian metapneumonovirus	~6 days	cotton fabric	yes	ss(-)RNA	Paramyxoviridae	Tiwari <i>et al.</i> , 2006
Low Pathogenicity Avian Influenza	~6 days	cotton fabric	yes	ss(-)RNA	Orthomyxoviridae	Tiwari <i>et al.</i> , 2006
Rat virus, (parvovirus, Yale strain)	>5 - 7 wks	corn cobb bedding	no	ssDNA	Parvoviridae	Yang <i>et al.</i> , 1995
Rabbit haemorrhagic disease	3.5 months	cloth	no	ss(+)-RNA	Caliciviridae	Smid <i>et al.</i> , 1991
Marburg virus (strain Popp)	4 - 5 days	stainless steel	yes	ss(-)RNA	Filoviridae	Belanov <i>et al.</i> , 1996
Avian metapneumonovirus	~6 days	steel	yes	ss(-)RNA	Paramyxoviridae	Tiwari <i>et al.</i> , 2006
Low Pathogenicity Avian Influenza	~6 days	steel	yes	ss(-)RNA	Orthomyxoviridae	Tiwari <i>et al.</i> , 2006
Avian metapneumonovirus	~48 hours	plastic	yes	ss(-)RNA	Paramyxoviridae	Tiwari <i>et al.</i> , 2006
Rat Coronavirus	2 - 3 days	6-well plastic plates	yes	ss(+)-RNA	Coronaviridae	Gaertner <i>et al.</i> , 1993
Marburg virus (strain Popp)	4 - 5 days	glass	yes	ss(-)RNA	Filoviridae	Belanov <i>et al.</i> , 1996
Low Pathogenicity Avian Influenza	~6 days	plastic	yes	ss(-)RNA	Orthomyxoviridae	Tiwari <i>et al.</i> , 2006
Rat virus, (parvovirus, Yale strain)	3 - 5 wks	plastic surface	no	ssDNA	Parvoviridae	Yang <i>et al.</i> , 1995

**Table 6**

Environmental stability studies on Coronaviruses.

Virus	Survival Time	Substrate	Authors
Human Coronavirus, serogroup OC43	3 hours	aluminum	Sizun <i>et al.</i> , 2000
Human Coronavirus, serogroup 229E	12 hours	aluminum	Sizun <i>et al.</i> , 2000
SARS Coronavirus	1 day	cotton gown, paper	Lai <i>et al.</i> , 2005
SARS Coronavirus	2 days	disposable gown	Lai <i>et al.</i> , 2005
Rat Coronavirus	2 - 3 days	6-well plastic plates	Gaertner <i>et al.</i> , 1993
Human Coronavirus (strain 229E)	3 days	polystyrene Petri dish	Rabenau <i>et al.</i> , 2005
SARS Coronavirus (isolate FFM-1)	9 days	polystyrene Petri dish	Rabenau <i>et al.</i> , 2005

Singapore, Taiwan and mainland China in September 2003, December 2003, and April 2004 (Lim *et al.*, 2006).

### Herpes Virus and B Virus

A special case of zoonotic virus warrants its own category because of the various uses of many species of primates in laboratory research. In 1932, Dr. W. B., a 29-year old researcher was engaged in experimental work on poliomyelitis when he was bitten on the dorsum of the left ring and little fingers by an apparently normal *Macacus rhesus* monkey. Sixteen days later he died (Sabin & Wright, 1934). Since this event, there have been several other LAI reports of B virus infection with some of these cases leading to death (Holmes *et al.*, 1990; Ostrowski *et al.*, 1998). B virus, or *Cercopithecine herpesvirus [CHV-1]*, is a member of the family Herpesviridae and, as such, is enveloped with a linear double-strand DNA that becomes circular in the host cell.

Members of the B Virus Working Group met at the Centers for Disease Control and Prevention (CDC) in January 1999 and formulated two recommendations. First, animal workers who care for macaques should be informed of the biohazards associated with these mon-

keys and the importance of notifying their supervisors and occupational health care personnel of all bites, scratches, and mucocutaneous exposures. Secondly, macaques should be treated as if they are seropositive for B virus regardless of their origin (Cohen *et al.*, 2002). The majority of studies on Herpesviridae species indicate a decreased viability on porous and non-porous substrates despite its DNA genome (Table 7).

### Discussion

Of the 177 pathogenic species regarded as emerging or re-emerging pathogens, the majority are viruses (77 species), followed by bacteria (54 species), fungi (22 species), protozoa (14 species), and helminths (10 species) (Zessin, 2006). Viruses will continue to be studied in the laboratory as researchers study new ways to ameliorate viral disease in humans, livestock, and plants. In addition to basic biological research, viruses are studied for vaccine and gene therapy development. Viruses can also be found in chronic disease research such as that addressing certain cancers caused by papillomavirus and herpesvirus.

**Table 7**

Environmental stability studies on Herpesvirus.

Virus	Survival Time	Substrate	Authors
Herpes Simplex Virus-1, strain F	<2 hours	coin penny	Bardell, 1994
Herpes Simplex Virus-1, strain F	>2 hours	chrome-plated tap handle	Bardell, 1990
Herpes Simplex Virus-1 and -2	18 - 24 hours	speculum	Larson <i>et al.</i> , 1985
Herpes Simplex Virus-1, strain F	>2 hours	glass	Bardell, 1994
Herpes Simplex Virus-1, strain F	>2 hours	plastic doorknob	Bardell, 1990
Cytomegalovirus	8 hours	plexiglass	Faix, 1985
Herpes Simplex Virus-1	1 d - 1 wk	glass slides	Mahl <i>et al.</i> , 1975
Herpes Simplex Virus-1 (strain McIntyre)	3 days	polystyrene Petri dish	Rabenau <i>et al.</i> , 2005
Cytomegalovirus	2 hours	blanket	Faix, 1985
Herpes Simplex Virus	>3 days	gauze	Larson <i>et al.</i> , 1985

The studies in this review indicate varying viral stability on porous (cloth) and non-porous surfaces (metal, glass, plastic) at typical laboratory relative humidity and temperature values. Within virus species, the retention of viability on metal surfaces is less than on glass or cloth substrates. This may indicate a possible virucidal mechanism of metals, as suggested by Bardell (1994), and supported by different survival times seen for the influenza virus on copper and steel (Noyce *et al.*, 2007). However, viability on surfaces is only half of the viral environmental stability equation. One must also look at viral stability while in aerosolized droplets. At least two viruses in this review, SARS and influenza, are readily transmitted by the respiratory route. Taken together, aerosolized and surface-laden viruses represent significant potential sources of infection in the laboratory environment.

As with all components of an institute's successful biological safety plan, education remains at the forefront of educating laboratorians how to protect themselves, as well as taking precautions to avoid infecting coworkers, including custodial staff. As viral environmental stability studies are presented to laboratorians, the awareness that some viruses have the ability to survive for days or weeks in the laboratory environment will be enhanced.

Further virus stability studies will allow for a more complete picture of other factors that influence environmental stability. In particular, little, or no research has been conducted on the stability of viruses affecting plants, an important consideration in agricultural biocontainment. Potential uses for these experiments, as suggested by Stuart & Wilkening (2005) include understanding degradation times of potential biological weapons with implications for time management during a terrorism response. Similarly, understanding the parameters required for virus breakdown in a contaminated lab, clinic room, or vivarium will enable the development and implementation of effective strategies to alter environmental conditions such as relative humidity and temperature, thereby avoiding costly decontamination procedures.

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