Viral Vector Biosafety

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Cambridge Biosafety Forum

May 9, 2023

Viral Vector

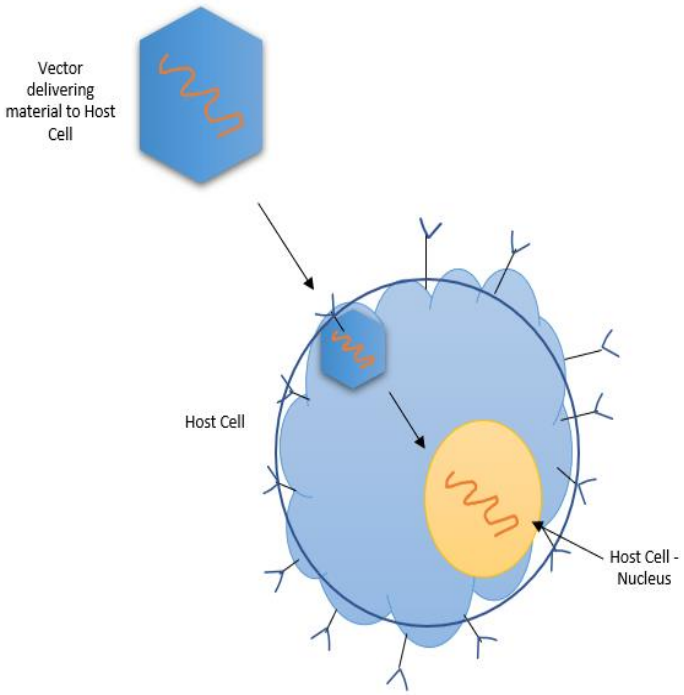
Viruses are naturally good at entering cells and delivering instructions (genetic code) to a host cell.

The host cell's machinery then expresses the viral genes. Using viruses as vectors allows scientists to take advantage of this feature to deliver and express genes of interest.

Viral vectors are constructed by replacing wild type viral genes with transgenes of interest.

The "useful" parts of the viral genome (delivery components) are isolated and used to make a vector while viral genes associated with virulence are usually removed.

The vector is usually safer than wild-type virus



Viral Vector - Uses

Basic research

- Delivery of genetic components to cell culture or research animals
- Can lead to expression of a gene of interest or expression of a regulatory component that may knock down or suppress gene expression

Gene therapy

- Delivery of a gene of interest to correct defective gene in a human patient or patient sample that is put back into the patient

Vaccines

- Delivery of pathogen genes into the body to stimulate an immune response

Vector Characteristics that affect Safety

Replication competency
- Viral vectors are often replication incompetent but not always
- Potential for reversion to a replication competent virus
- Consider the risk level of the wild type virus

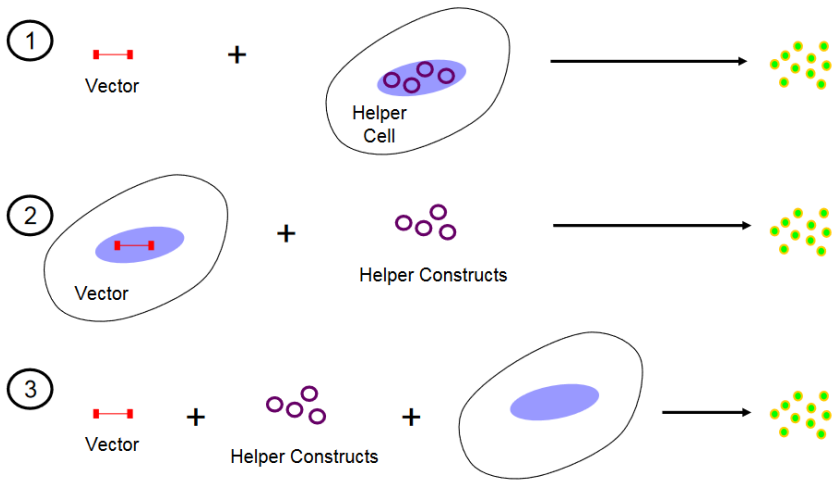
Tropism
- Host range of the vector determines the species/cell type that the virus can enter

Integration potential
- Integration of the viral vector genome into the host genome can lead to insertional mutagenesis

Transgene hazard
- Function of the gene “payload” carried by the vector

Gene regulation
- Components regulating expression of the transgene, where, when

Replication Competency



When making a viral vector, as many viral genes as possible are removed from the viral genome

- Many viral genes are missing when the virus enters a host cell and thus the virus carries no instructions for how to replicate itself and spread to neighboring cells
- Separation of structural from sequence to be packaged

But how to you produce a viral vector such as this if the instructions for making itself are gone?

- Helper function provided during packaging
- Provides opportunity for recombination of replication genes into vector genome

Tropism

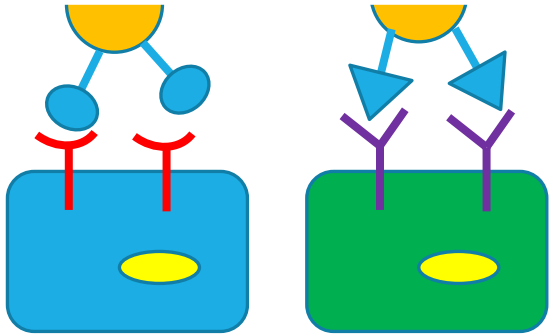
The specificity of a virus for a particular host tissue, determined in part by the interaction of viral surface structures with host cell-surface receptors.

Ecotropic: Only able to infect rodent cells

- Safest option, ecotropic lentiviral systems available

Amphotropic: Cell envelope has been modified to also infect most mammalian cells (possibly human)

Pantropic VSV-G pseudotyped: Cell envelope has been modified to infect many other tissue types and species, definitely human



Integration

Some viral vectors can integrate their genome into the host cell's genome

- This can be a desirable trait for long-term expression of a transgene

There are also risks involved

Insertional mutagenesis

- Oncogenesis (development of cancer) due to disruption of a tumor suppressor
- Dysregulation of host genes due to regulatory elements present on the viral genome (enhancement of host oncogenes nearby the insertion site)

Mobilization of the vector from the genome of transduced cell

- Complementation/helper function in individuals infected with wild type virus (example: lentivirus and HIV+ individuals)

Transgene Hazard and Regulation

In addition to assessing the risk of the viral vector to be used, you must think about the transgene or recombinant nucleic acids that will be carried by the vector

What functions are associated with this transgene and how might they affect a researcher if accidentally exposed?

Does expression or function of these genes require any other elements that aren't carried by the vector?

- Example: CRISPR system – vector with gRNA and Cas9 vs. separation of these elements

What expression control elements are in place?

Does the promoter drive a high level of expression or is it restricted to a certain cell type?

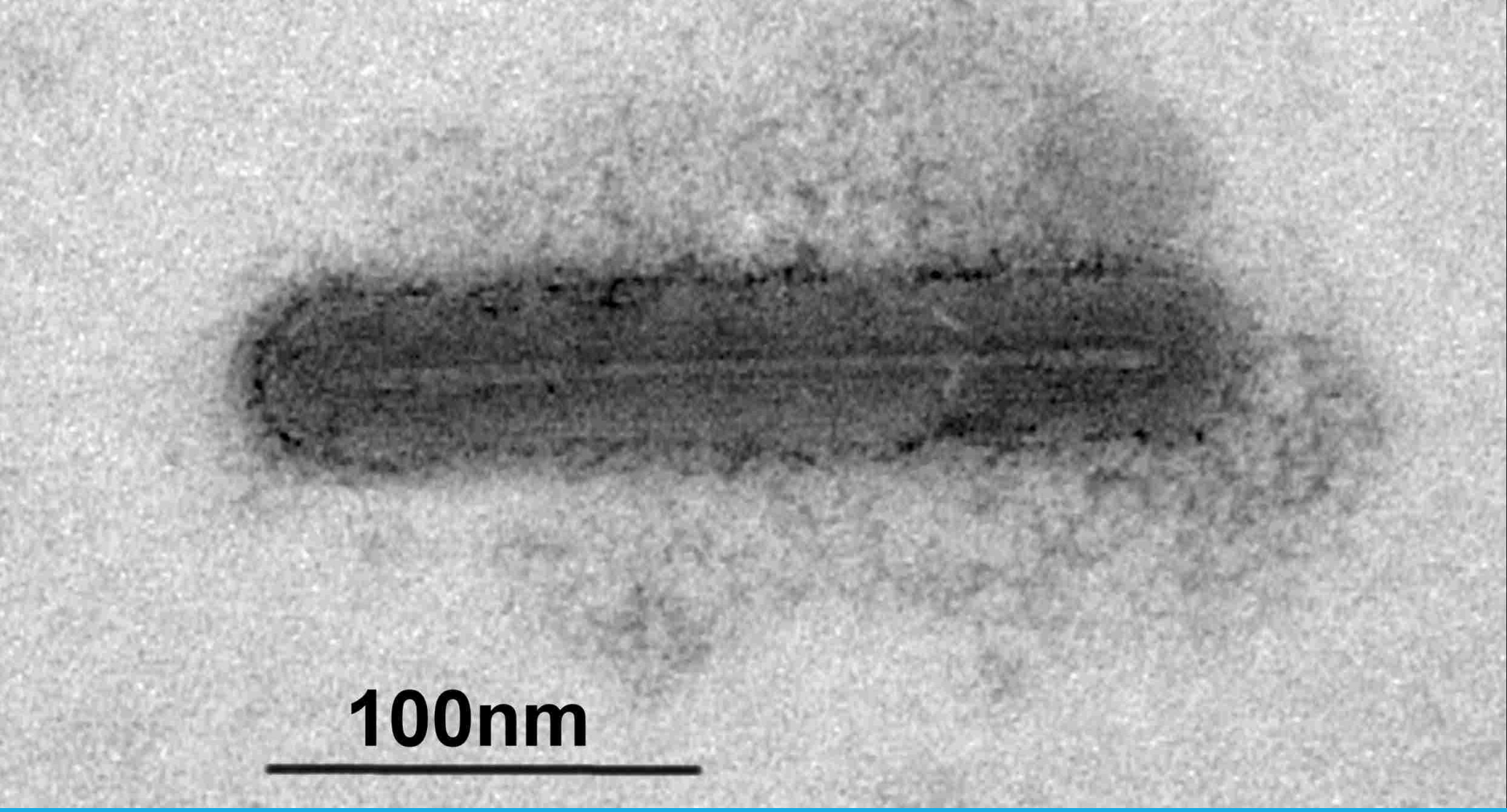
Is expression or function inducible or dependent on other factors?

Are these factors present in a research who may be exposed?

Viral Vector Risk Assessment

Things to consider:

- Risk of the wild type virus
- Replication competency
- Tropism
- Integration potential
- Transgene function
- Gene regulation
- Susceptibility to disinfection
- Permissive animal host



Baculovirus

Baculovirus

Large, rod-shaped viruses

Enveloped

Host range is invertebrates, usually insects

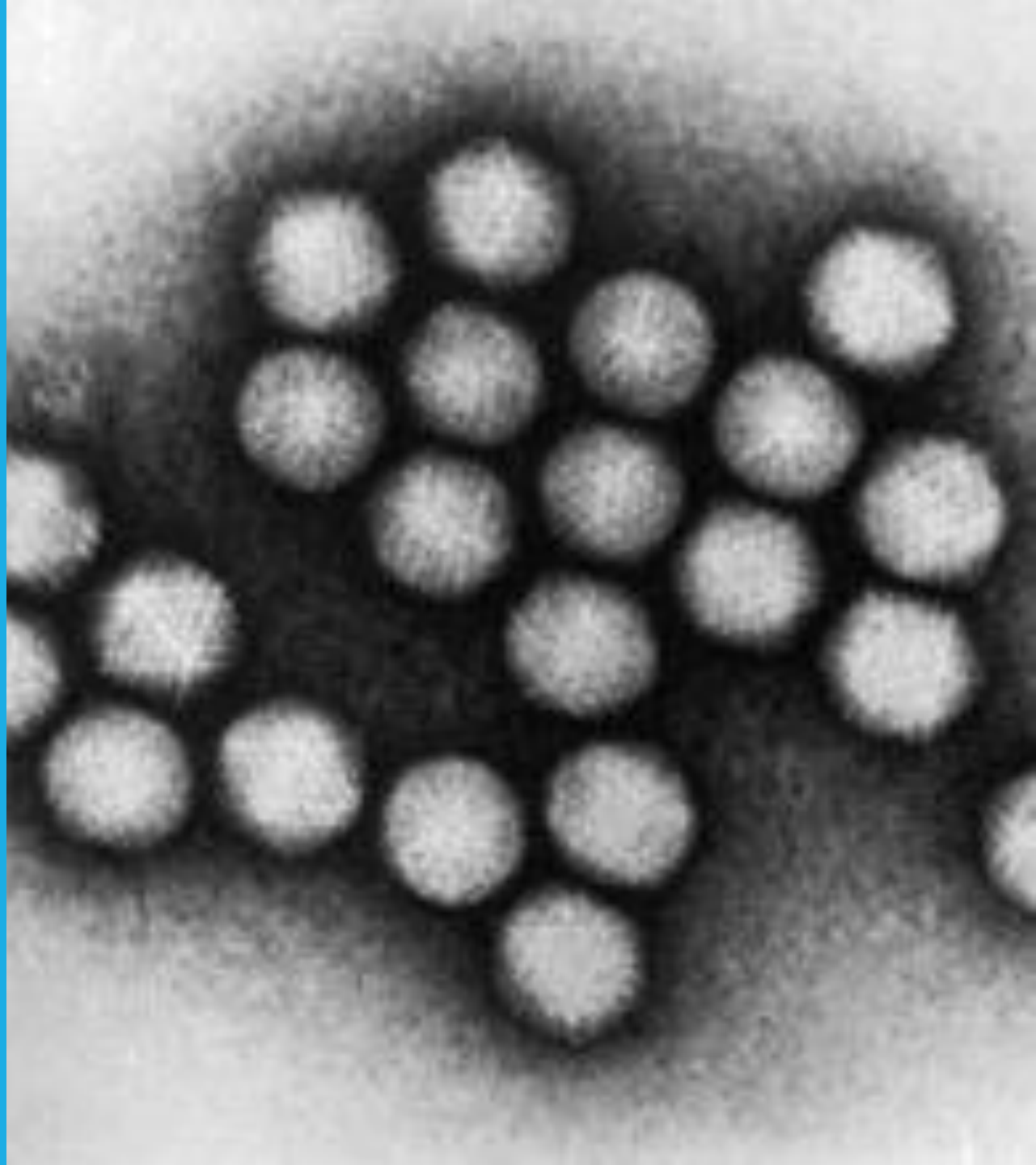
May be managed at BSL-1

Testing for replication-competent virus is not required

Even though non-pathogenic to humans decontamination must still be performed

- Susceptible to 70% EtOH
- Standard autoclaving

Adenovirus



Adenovirus

Non-enveloped, icosohedral virus, 57 serotypes

dsDNA genome

5-10% of upper respiratory infections in children

Modifications to fiber proteins allow delivery to specific targets

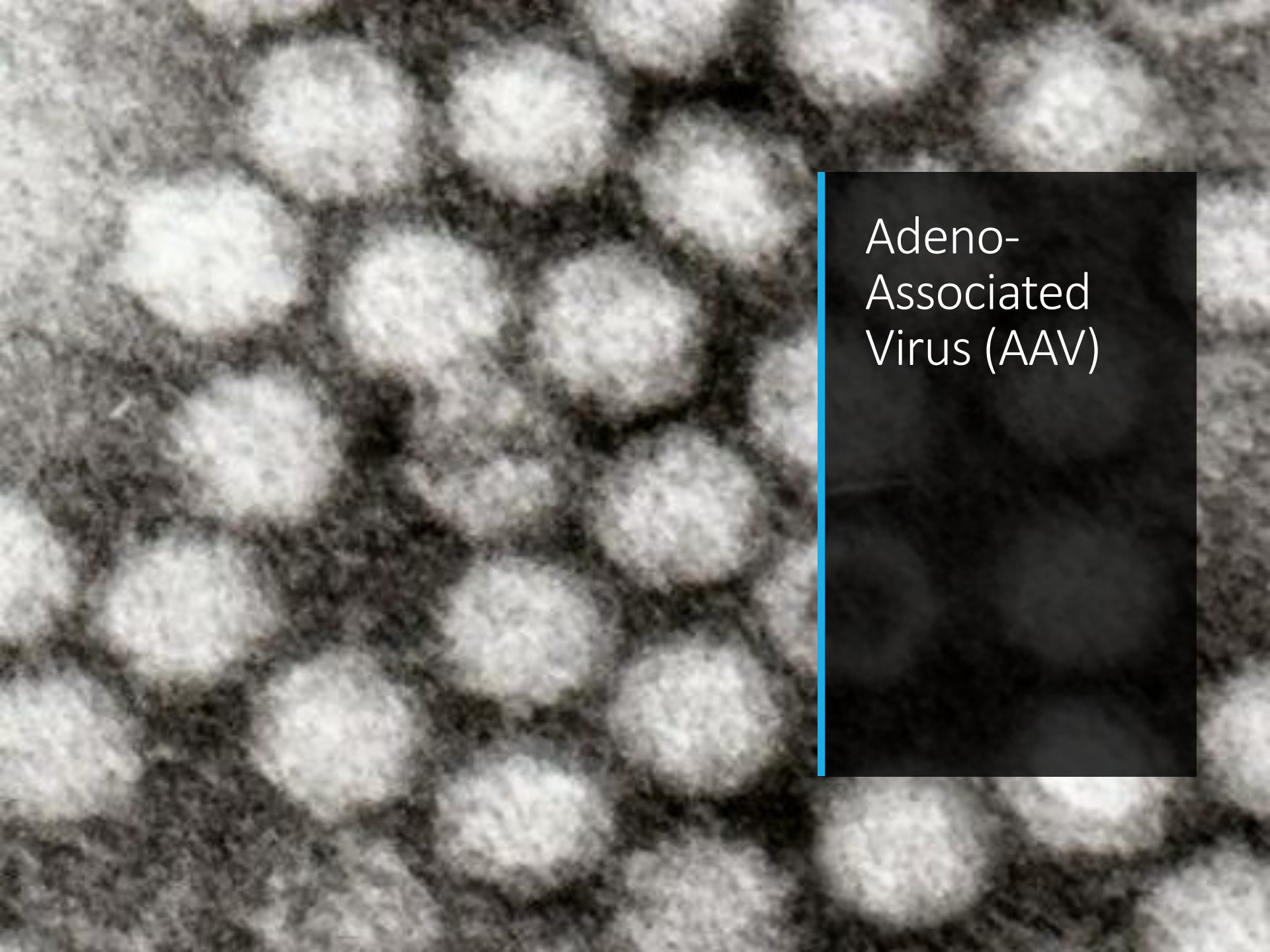
Usually does not integrate into the genome

Should be managed at BSL-2

- Oral contact or viral spread on hands, paper, etc
- Very robust in the environment because there is no envelope
- Decontamination of surfaces and hand-washing

Decontamination

- Since there is no envelope, 70% EtOH is not effective
- 10% bleach, followed by 70% EtOH (to help eliminate residual bleach)
- Standard autoclaving


Adeno-
Associated
Virus (AAV)

Adeno-Associated Virus (AAV)

Very small (20 nm), Non-enveloped

ssDNA genome

Naturally replication incompetent (requires Adenovirus to replicate)

Infects humans, but not known to cause disease

Possible link to male-infertility, although no causal relationship is established

NIH lists as Risk Group 1 (not known to cause disease in healthy adults)

Integrates at a very low frequency

Decontamination

- Since there is no envelope, 70% EtOH is not effective
- 10% bleach
- Standard autoclaving

AAV and Helper Virus

VECTOR ALONE (NO HELPER)

When used as a vector alone (without adenovirus helper)

BSL-1 should be used

If administered to animals, ABSL-1 is sufficient

No testing for replication-competent virus is required

WITH HELPER VIRUS

When used in conjunction with adenovirus

The same guidelines as using adenovirus apply

BSL-2

AAV as a Gene Therapy Vector

Wild type virus is considered non-pathogenic RG1

Near complete removal of viral coding sequences

- Maximizes packaging capacity of vector
- Contributes to low immunogenicity and cytotoxicity in vivo

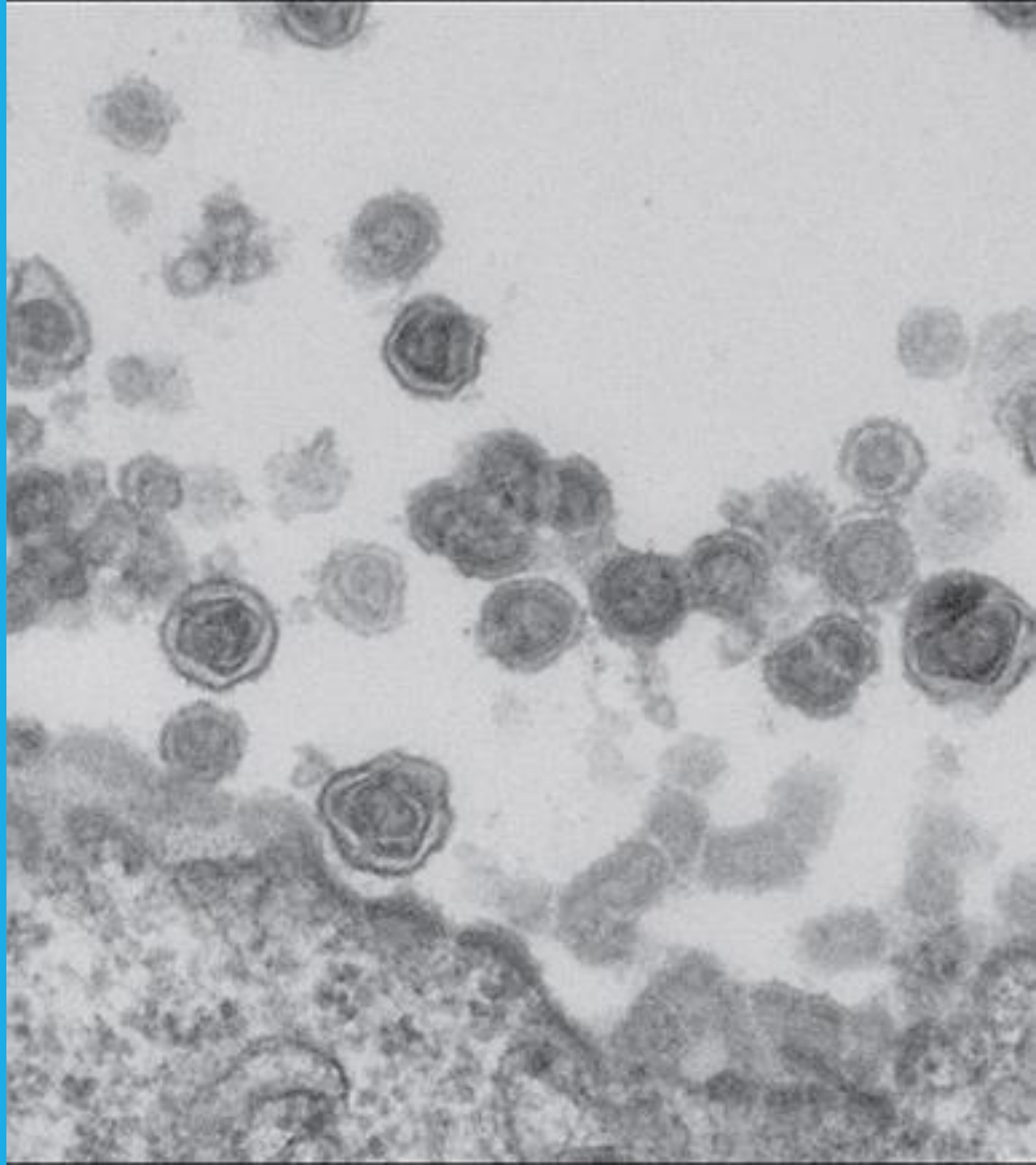
Genome limitations to approximately 5.0 kb

Different serotypes have different tissue-type and cell-type tropisms

Persistent expression in postmitotic cells

2012 – First FDA approval of an AAV-based gene therapy drug

Murine Retrovirus



Murine Retrovirus

Retroviruses that infect rodents in nature

Enveloped

RNA genome, requires reverse transcription to DNA (reverse transcriptase)

Ex: Moloney Murine Leukemia Virus (MMLV)

Infects dividing cells only

Can integrate into the genome to cause cancer due to insertional mutagenesis

Ecotropic, amphotropic, pantropic

For decontamination:

- Envelope makes the virus labile and sensitive to many disinfectants, including 70% EtOH or 5%-10% bleach solution

Murine Retrovirus Tropism

ECOTROPIC

Only able to infect rodent cells

May be managed at **BSL-1**

Testing for replication-competent viruses usually not required by IBC

Can be oncogenic for live rodents

When administered to animals, ABSL-1 is sufficient

Safest option but has research limitations

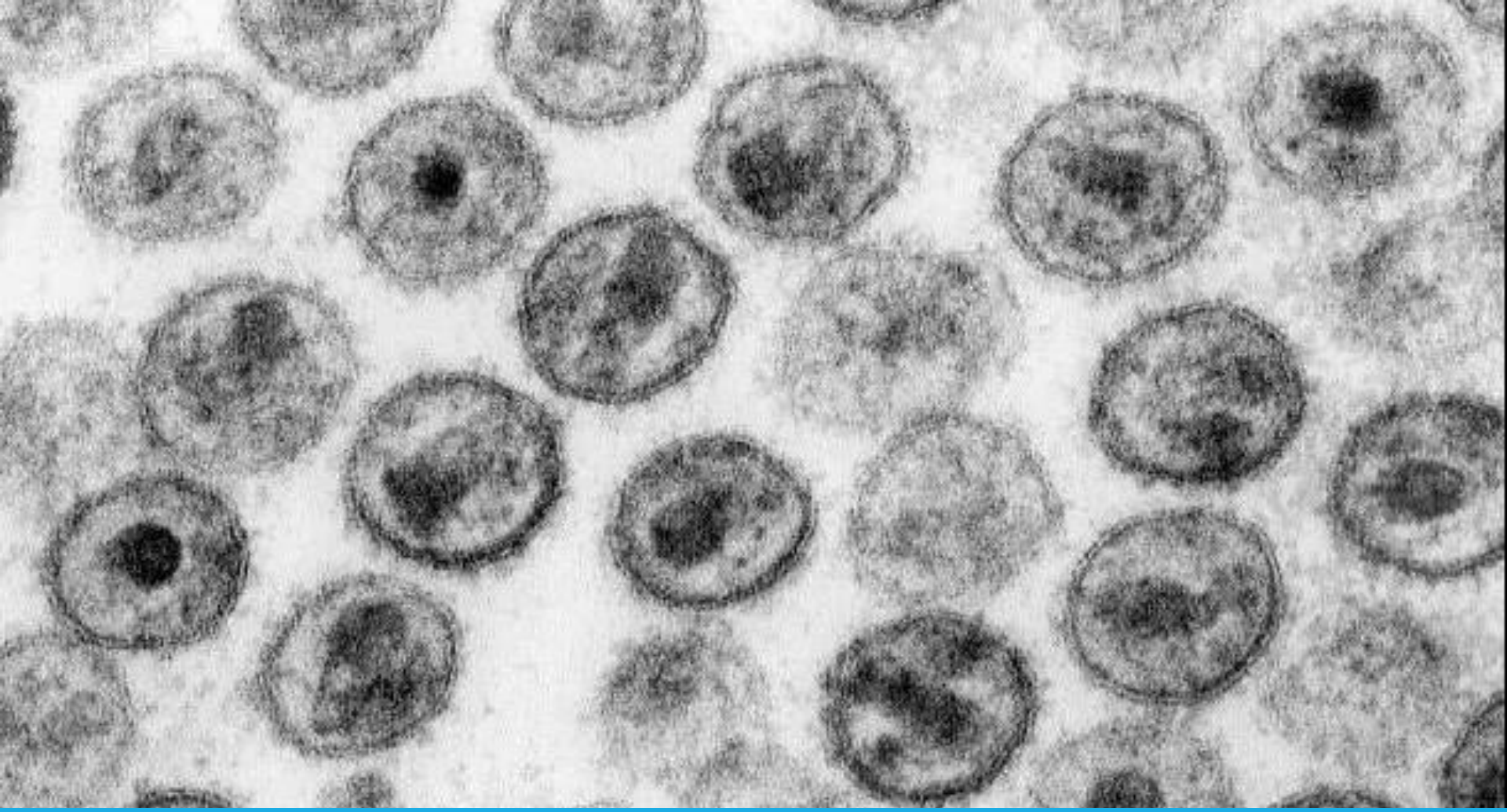
AMPHOTROPIC OR VSV-G PSEUDOTYPED

Cell envelope has been modified to also infect other mammalian cells (possibly human).

Should be managed at **BSL-2**, due to expanded host range

Testing for replication-competent virus may be required by IBC

If administered to animals (provided no replication-competent virus is detected), ABSL-1 is sufficient for housing/handling of animals.



Lentivirus

Lentivirus

Derived from HIV

Enveloped retrovirus

RNA genome

Able to infect human cells

Can infect dividing and non-dividing cells

Readily integrates into the genome, possibly with oncogenic effects

Transmitted by:

- Direct inoculation: Needle stick, entry through wound, etc.
- Mucosal exposure (eyes, mouth, nose)
- No data to suggest respiratory transmission

Lentivirus Risks

Production of replication competent virus

Oncogenesis from insertional mutagenesis

Mobilization of virus from the genome of transduced cell

Biosafety Considerations for Lentivirus

Biosafety Consideration	Lower Risk Example	Higher Risk Example
Vector generation	4-plasmid system (3rd gen)	3 plasmid system (2nd gen)
Self-inactivation	SIN	Non-SIN
Tropism	Non-human, ecotropic	Broad host range (VSV-G)
Transgene function	GFP	"high risk genes" (tumor suppressor, oncogene)
Expression control elements	Weak or inducible promoters	Strong promoters (CMV, SV40)
Scale	Lower viral concentrations, volumes	Higher concentrations, volumes
Animal host	Non-permissive host	Permissive host (humanized mice)
Procedures	No sharps	Sharps use

Adapted from: Schlimgen R, Howard J, Wooley D, et al. Risks Associated With Lentiviral Vector Exposures and Prevention Strategies. *J Occup Environ Med*. 2016;58(12):1159-1166. doi:10.1097/JOM.0000000000000879

Lentivirus

Often used at BSL-2 or BSL-2 w/ BSL-3 practices (a.k.a., BSL2 enhanced, BSL2+)

- All work done in biosafety cabinet (BSC)
- Minimize (and if possible, eliminate) use of sharps (needles, scalpels, glass)
- Mucous membrane protection
- Double gloves?
- Lab coat

Decontamination

- Envelope makes lentivirus susceptible to 70% EtOH
- 10% bleach
- Standard autoclaving

IBC Considerations for Viral Vectors

Many of these vectors are available as kits through vendors

- Researchers may need to seek information from the vendor/source in order to answer pertinent IBC questions
- Modern vector system are becoming increasingly complex, researcher may not be aware of every safety detail

Be confident in the answers to each aspect of the viral risk assessment. Don’t make assumptions.

There are many viruses that can be used as vectors:

- Murine retrovirus, herpesvirus, lentivirus, adenovirus, adeno-associated virus, alphavirus, baculovirus, poxvirus, rabies virus