Using Viral Vectors in Animal Research

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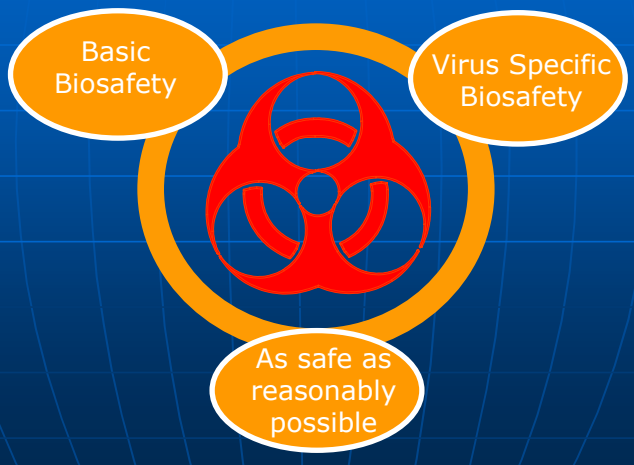
ChABSA Symposium
June 8, 2006

The Rules Should Follow Biology and Common Sense



Basic Biosafety Concerns

- What is the pathogenicity of the parental virus?
- What is the host range of the parental virus? (infection vs. replication) and has anything been done to extend the host range of the vector?
- Has anything been done to extend the pathogenicity? (oncogenes, toxin genes, etc.)
- Is the isolated DNA infectious?
- Can the recombinant DNA be mobilized? (viral vs. nonviral DNAs…complementation and recombination)
- Is recombination an issue? (is all the virus there, albeit in separated genetic elements?)

Virus Specific Biosafety Concerns

- What is the normal route of infection? (aerosols vs. direct contact)
- Can the vector interact with endogenous viruses? (MLV vectors in murine cells)
- Can the vector interact with exogenous viruses (human adenoviruses with adenovirus and AAV vectors)
- If the vector is intended to be defective are there any replication competent recombinants in the stock?
- Does the disinfectant/procedure inactivate the vector that is being used?

As Safe as Reasonably Possible

- Biological barriers are your best protection: If the vector won't replicate in a human……
- Physical barriers (biological safety cabinets, gloves, masks, clothing, caging, etc.) are important, but they need to match the route of infection.
- Watch out for sharps/needles!
- Your immune system is the final level of protection; try not to use it (vaccination can help in some cases).
- Know what you are working with: Quality control for cells, animals and vectors.

Who Are We Protecting?

- Research laboratory staff/animal husbandry personnel
  - When the vector is introduced into the animal.
  - Care and husbandry of infected animals.
  - When infected material returns to the research laboratory.
- Animals in the colonies

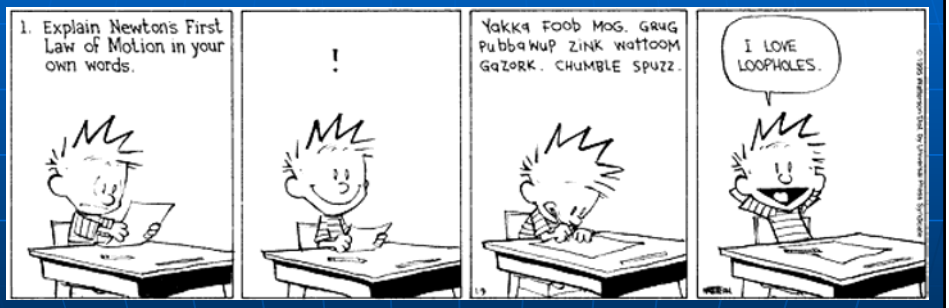
How Is an Animal Different from a Petri Dish?

- Eating, Excreting
- Biting, sneezing
- Confining the inoculum
- Sharps: injections and dissection of tissues
- Disposal of infected animals and bedding
- Animal handlers (informed consent)

What the IBC Should Know

- What experiments will be done with recombinant DNA and/or viral vectors?
- What viral vectors will be used?...Molecular details can be important…
- Will anything be done that would extend the host range or enhance the pathogenicity of the vectors?
- Is it reasonable to expect that the vectors can be complimented or recombine in the proposed experiments?
- What will be done to minimize the risks in the proposed experiments?

A PI Fills Out IBC Forms….



IBC Forms and Responses

- The form should ask the right questions
- The form should provide some guidance, but not specific answers
- What won't be done in the experiments can be as important as what will be done
- What should the IBC do when the PI doesn't understand the problem?
- What about reagents/animals generated elsewhere (commercial and noncommercial sources)?
- How are transgenic animals tracked and what are the concerns?

The IBC : ACUC Interface

- Interaction between the two committees needed for comprehensive safety program
  - Sharing animal use protocols between committees
  - ACUC forms have questions about rDNA use
- Co-mingled memberships between the two committees leads to consistency in review process

Expression of Foreign Genes in Animals

- Recombinant DNA (rDNA) techniques can be used to obtain expression of a foreign gene or genes
- DNA integrates: non-viral transgenic technologies, retroviruses, AAV
- DNA is not (normally) integrated: Poxvirus, adenovirus, herpesvirus

The NIH Guidelines and Beyond

- What the NIH Guidelines tell us
- Viruses and viral vectors
- A common sense approach
- What could go wrong? Imagining the worst case scenario

Risk Groups Described in NIH Guidelines

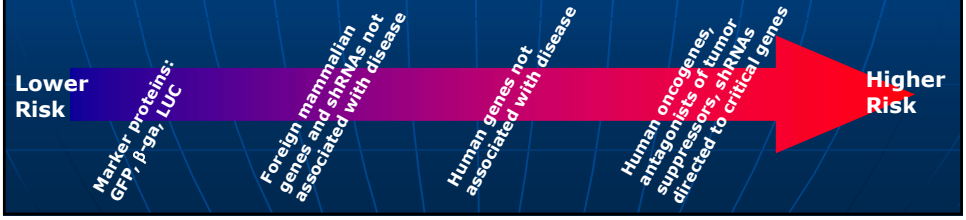
Risk Group 1 (RG1)	Agents that are not associated with disease in health adult humans (AAV [helper-free] and MuLV with benign transgenes)
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available (Adeno-, Hepes- and Poxviruses & recombinants)
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may be* available (high individual risk but low community risk) (HIV-1 &-2, SIV, HTLV-1 & -2)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

Properties of Viral Vectors

Virus	Insert Size	Persistence	Advantages	Disadvantages
Retrovirus	1-8 kb	Permanent	Stable integration	May rearrange genome, may require cell division
Adeno-associated virus	~4 kb	Transient	Prolonged transient	Limited capacity, current vectors nonintegrating
Adenovirus	2-35 kb	Transient	Efficient gene delivery	Transient, neurons, very high titers
Vaccina virus (poxvirus)	~25 kb, probably more	Transient	Wide host range, ease of isolation, large capacity, high expression	Transient, immunogenic, morbidity of infct

Developing a Broader Sense of Risk for rDNA and Viral Vectors

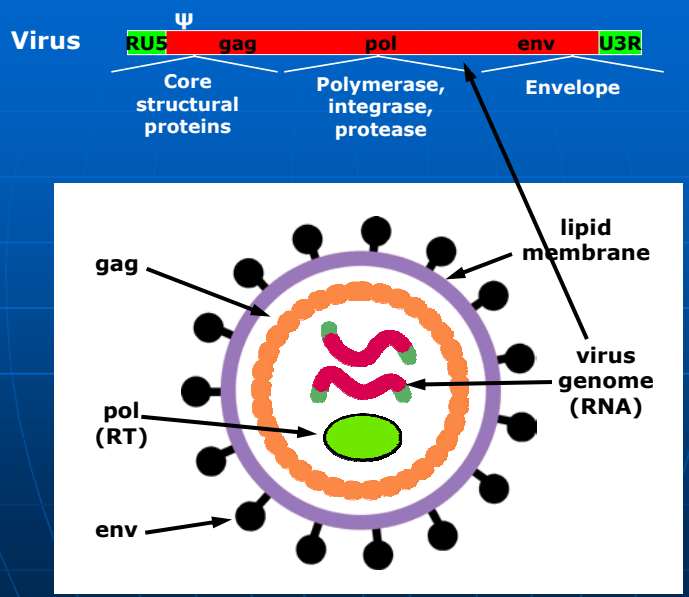
- Biological control
- Biological function of transgene
- Immunity for viral vector
- Immunity evoked by transgene
  - Human genes
  - Regulatory RNAs



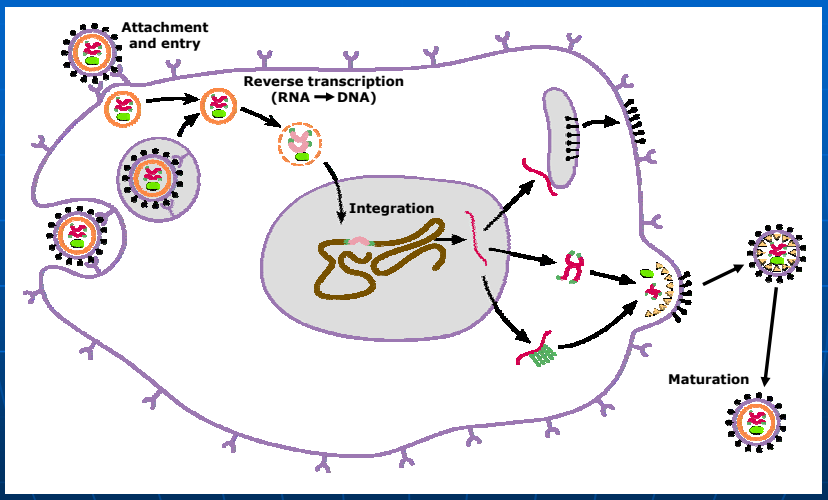
Special Considerations for Retroviral Vectors

- Retroviral DNA integrates into the host cell genome: Infections can persist, and the insertions are mutagenic
- MLV insertions can cause tumors in non-human primates and in immunosupressed humans
- Retroviruses are highly recombinogenic: If the vector is supposed to be replication defective, make sure that it is
- MLV vectors can recombine with endogenous viruses in murine cells
- HIV is a significant human pathogen

Retrovirus Structure



Retrovirus Replication Cycle



Unmodified retroviruses are "Replication Competent" (production of progeny viruses that are infectious)

Making a Retroviral Vector from a Retrovirus

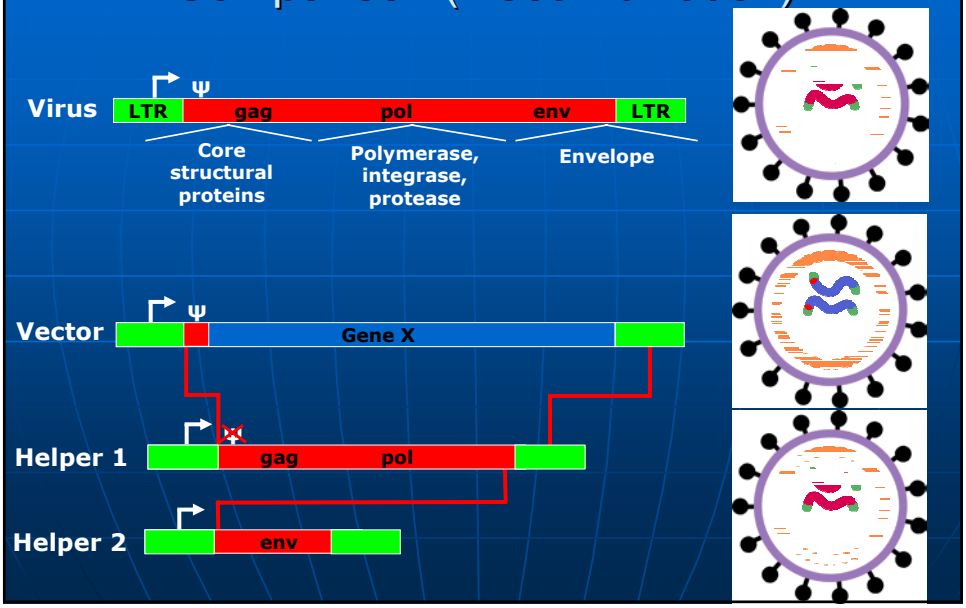
Properties of a retroviral vector:

1. Express a gene or genes not normally found in the retrovirus.
2. After infection of host, some or all of the retroviral genes are not expressed.
3. In the host, the retroviral vector should be replication incompetent (potential problems with recombination and, for MLV, endogenous retroviruses).

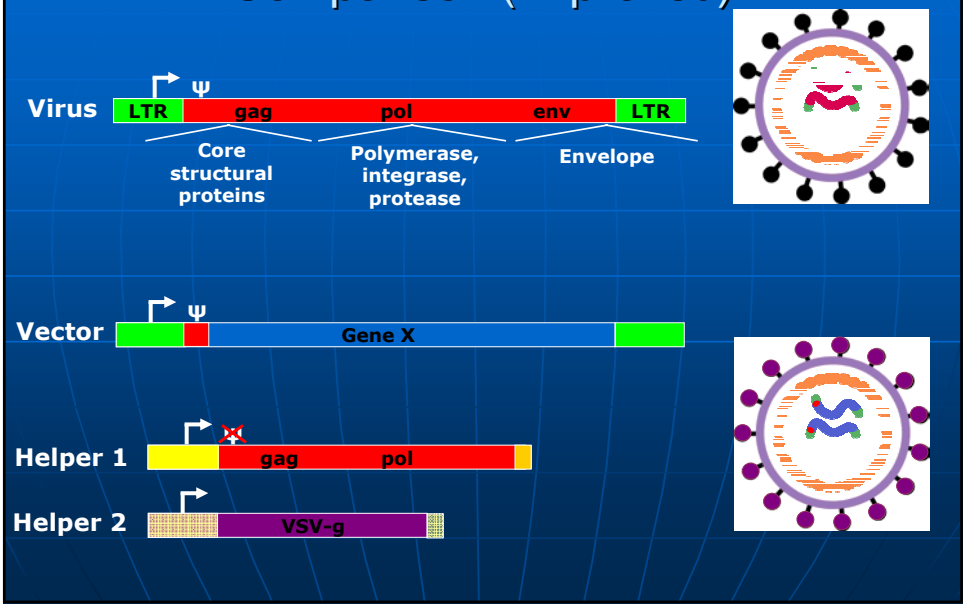
How a defective retroviral vector is made:

1. Some or all of the structural genes needed for viral replication are removed from the genome.
2. Gene(s) of interest (Gene X) are inserted.
3. Structural genes are supplied in trans as separate genetic elements.

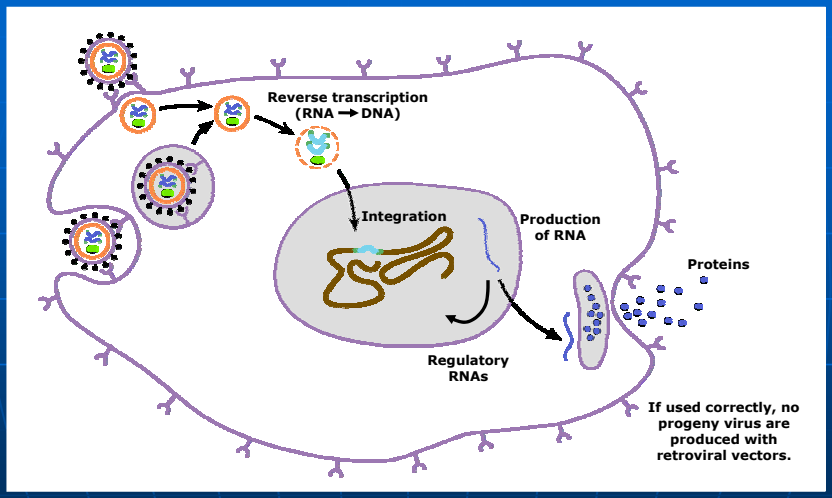
Retrovirus and Retroviral Vector Comparison (Recombination)



Retrovirus and Retroviral Vector Comparison (Improved)



Retroviral Vector Infection



Retroviral vectors are designed to be "Replication Incompetent" (doesn't make more infectious progeny)…but only if manufactured correctly.

Special Considerations for Vaccinia Vectors

- Many vaccinia vectors are replication competent
- Vaccinia is readily transmitted to a variety of mammals, including humans
- Vaccinia vectors can carry a large insert, and can be used to enhance the host range of pathogenic viruses
- Titers to 10^{10}
- Vaccination can be used to reduce lab worker susceptibility

Special Considerations for Adenovirus Vectors

- Adenoviruses are highly recombinogenic
- Vector stocks that are supposed to contain only defective vectors may contain replicating viruses
- Lab workers may harbor replicating adenovirus that can compliment a defective vector
- Vectors that have an extended host range have been developed
- Very high titers: 10^{12}

Recombination

- Are all the sequences needed to reconstitute the virus ever present in one cell?
- Sequence homology enhances the rate of recombination but recombination still happens in the absence of homology.
- Rare events happen frequently in high titer viral stocks.
- It only takes one replication competent recombinant virus.

DISPATCHES

Ocular Vaccinia Infection in Laboratory Worker, Philadelphia, 2004

Felicia M.T. Lewis,*† Esther Chernak,* Erinn Goldman,† Yu Li,† Kevin Karem,† Inger K. Damon,† Richard Henkel,† E. Claire Newbern,* Patrina Ross,* and Caroline C. Johnson*

We report a case of ocular vaccinia infection in an unvaccinated laboratory worker. The patient was infected by a unique strain used in an experiment performed partly outside a biosafety cabinet. Vaccination should continue to be recommended, but laboratories with unvaccinated workers should also implement more stringent biosafety practices.

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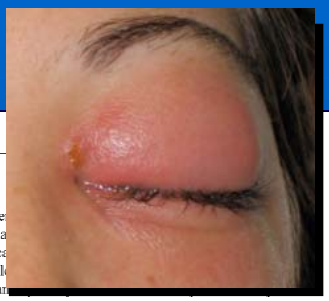
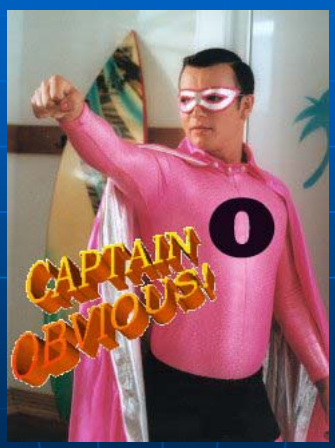
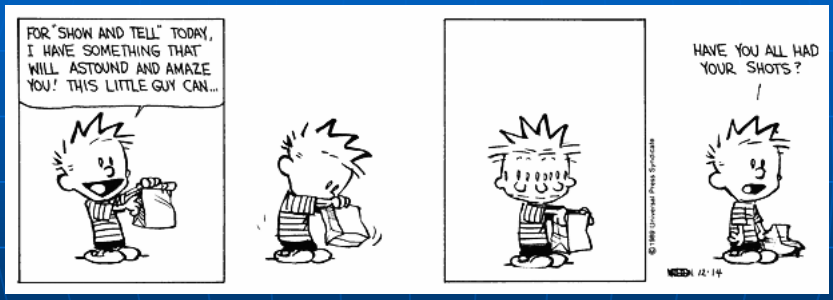


Were laboratory practices followed…



Would Vaccination Help?

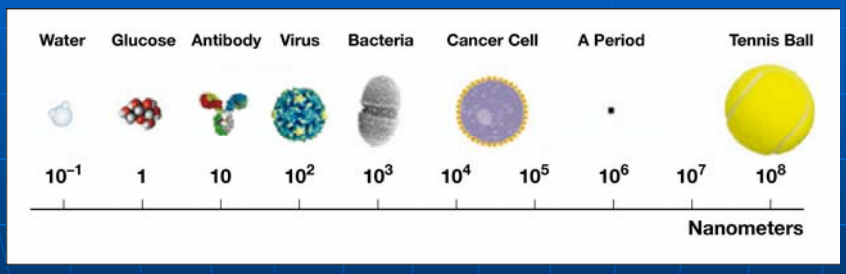


Quality Control: Are You Sure You Know What You Are Getting?

Viral Vector Quality

- What to monitor
  - cDNA constructs
  - Producer cells
  - Viral vector stocks
  - Animals
- What to monitor for:
  - Is it the right vector?
  - Replication competent recombinants
  - Endogenous contaminants

Size Matters



It is difficult to track things that are very small.

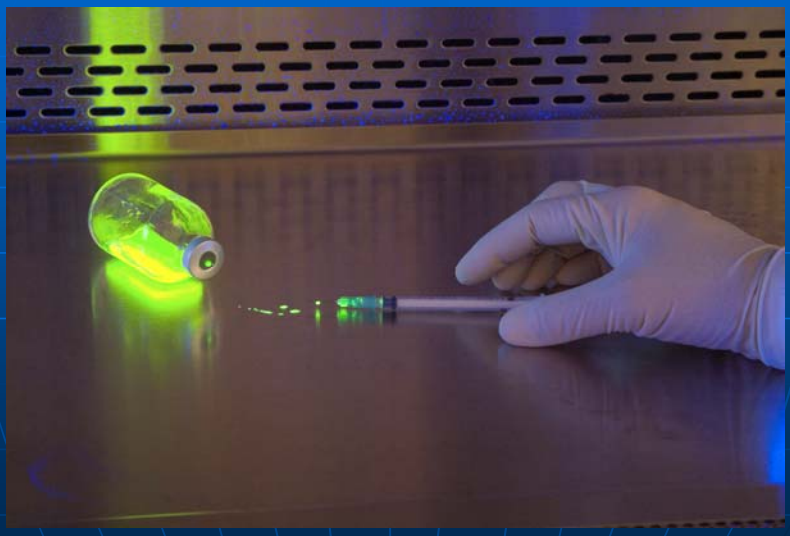
Do a Test Run with a Fluorescent Marker

- Fluorescent materials for tracking materials prior to use with live agent
- Easily tracked with UV light
  - Illumination from a UV light in safety cabinet/hood
  - Hand-held UV light
- Markers:
  - Riboflavin
    - 200mg/L
  - Fluorescein
    - 350mg/L

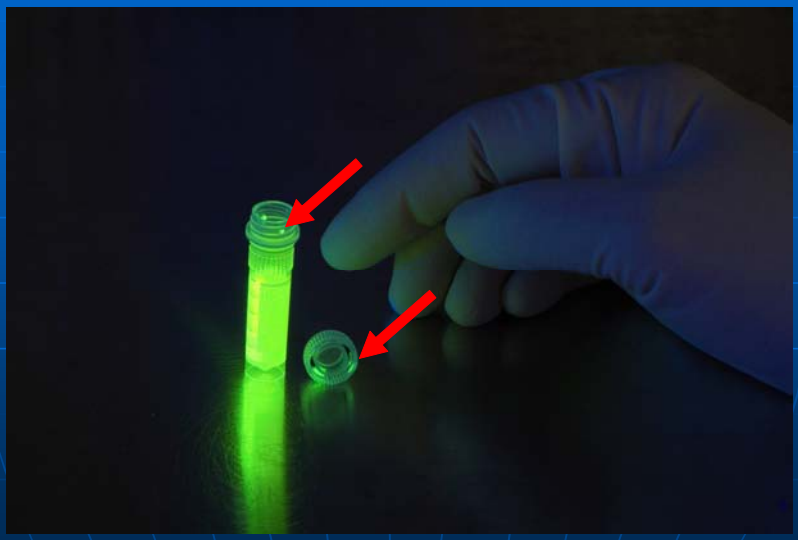
Riboflavin and Fluorescein

Fluorescein photobleaches after exposure to ultraviolet light

Spray from Needle/syringe



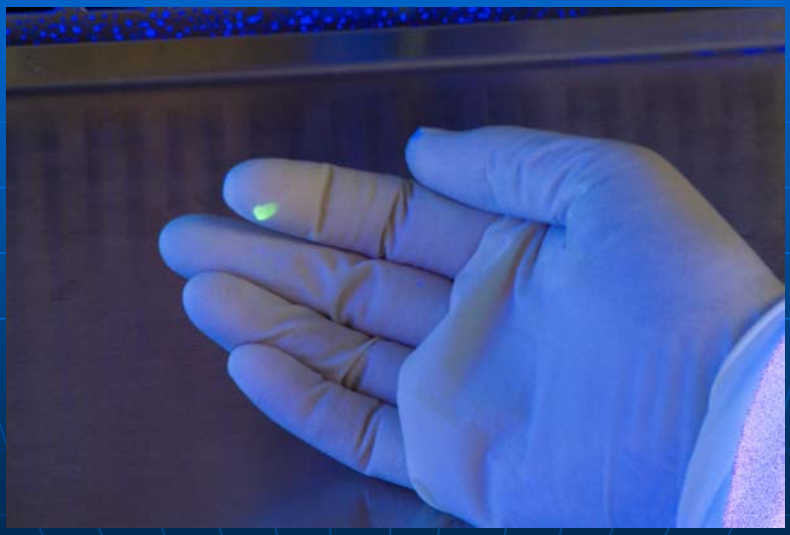
Material Retained on Threads of Cap



Transfer materials to a clean tube to eliminate contamination

Where's the Spill?

UV Light Exposure



Importance of Gloves

- Changing gloves to reduce bio-burden
- Decontaminate gloves if needed before removing
- Consider trade offs of working while double gloved

Restraining, Injecting and Caging Mice

- Injecting virus into animals…inject the animal….not yourself
- Appropriate injection technique
- Restrain animals:
  - Chemical or physical
- Proper caging

Mouse Restraint

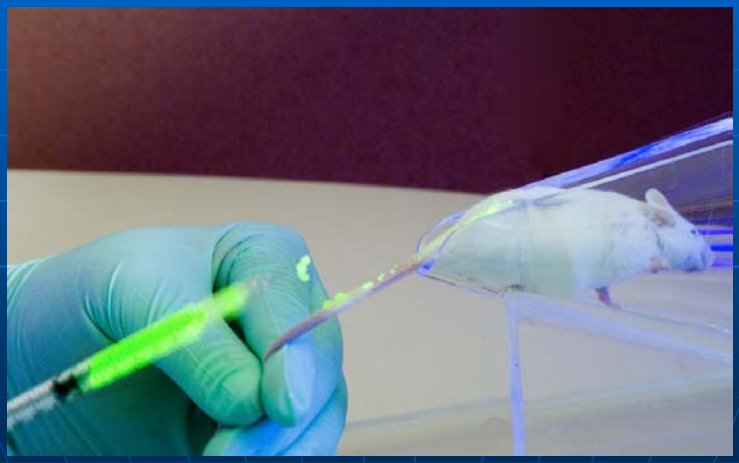
- Physical

- Chemical

Restraint Device

- Hands away from action…

Post-Injection Leakage



Injections

- Hand more likely to have needle contact
- When possible, position animal such that the needle isn't in-line with hand

Caging

- Microisolator

Acknowledgements:

- Steve Hughes
- Joe Kozlovac
- Dexter Poon
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- Julie Bullock
- Misty Hawes