Institutional Biosafety Committees:
Promoting Optimal Practice
Now and in the Future

Assessing the Risks of Viral Vector Protocols

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Advanced Technology Program

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Outline

- Viral Vector Protocol Review:
  - What to look for
  - How to make it safe
- Role of the IBC
  - NIH Guidelines for Research Involving Recombinant DNA
- How to assess risk
  - The registration form
  - What the IBC needs to know
  - The underlying biology of the virus/vector system and transgene being expressed
  - Quality assurance requirements

Outline Continued

- Mitigation of risks
  - Process controls
  - Training & Testing
    - Fluorescent markers and their application
  - Engineering controls
  - PPE
  - Vaccination
  - Quality control
    - Virology and Molecular Biology tool kit
- Animals: Added Risks

Role of the IBC

- To obtain a full understanding of the risk associated with viral vector research
- To provide a comprehensive review and independent risk assessment of the research
  - Subject matter experts
- To ensure that experiments are conducted safely, and that appropriate measures are used to mitigate risks

NIH Guidelines: Risk Groups

Risk Group 1 (RG1) Agents that are not associated with disease in health adult humans

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

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)

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)

Appendix B-I. Risk Group 1 (RG1) Agents

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Adeno-associated virus (AAV) types 1-4

Recombinant AAV with benign transgene and without helper virus contamination

MuLV and MuLV recombinants encoding benign transgenes

Risk Groups Described in NIH Guidelines

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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)

Appendix B-II-D. Risk Group 2 (RG2) – Viruses

Adenoviruses, human - all types

Alphaviruses (Togaviruses) - Group A Arboviruses

–Eastern e

–Venezuel
strain TC-83

Flaviviruses (Togaviruses) - Group B Arboviruses

–De

–Yel

Hepatitis A, B, C, D, and E viruses

Herpesviruses - except Herpesvirus simiae (Monkey B virus)

–Cytomegalovirus

–Epstein Barr virus

–*Herpes simplex* types 1 and 2

–*Herpes zoster*

–Human herpesvirus types 6 and 7

Orthomyxoviruses

–Influenza viruses types A, B, and C

Papovaviruses

–All human papilloma viruses

Paramyxoviruses

–Newcastle disease virus

–Human parvovirus (B19)

–Rhinoviruses - all types

Poxviruses - all types except Monkeypox virus and restricted poxviruses including Alastrim, Smallpox, and Whitepox

Reoviruses - all types including

Rhabdoviruses

–Rabies virus - all strains

–Vesicular stomatitis virus – laboratory adapted strains including VSV-Indiana, San Juan, and Glasgow

Togaviruses (see Alphaviruses and Flaviviruses)

–Rubivirus (rubella)

Adenoviruses, human—all types

Herpesviruses—except Herpesvirus simiae

Poxviruses, except restricted groups

Risk Groups Described in NIH Guidelines

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Appendix B-III-D. Risk Group 3 (RG3) Viruses and Prions

Alphaviruses (Togaviruses) - Group A Arboviruses

–Semliki Forest virus

–Venezuelan equine encephalomyelitis virus (except (RG2))

Flaviviruses

–Japanese

–Yellow fe

Poxviruses

–Monkeyp

Retroviruses

–Human immunodeficiency virus (HIV) types 1 and 2

–Human T cell lymphotropic virus (HTLV) types 1 and 2

–Simian immunodeficiency virus (SIV)

Rhabdoviruses

–Vesicular stomatitis virus

Retroviruses

- HIV-1 and HIV-2
- HTLV-1 and HTLV-2
- SIV

Risk Groups Described in NIH Guidelines

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Appendix B-IV-D. Risk Group 4 (RG4) Viral Agents

Arenaviruses
- –Guanarito virus
- –Lassa virus
- –Junin virus
- –Machupo virus
- –Sabia

Bunyaviruses (Nairovirus)
- –Crimean-Congo hemorrhagic fever virus

Filoviruses
- –Ebola virus
- –Marburg virus

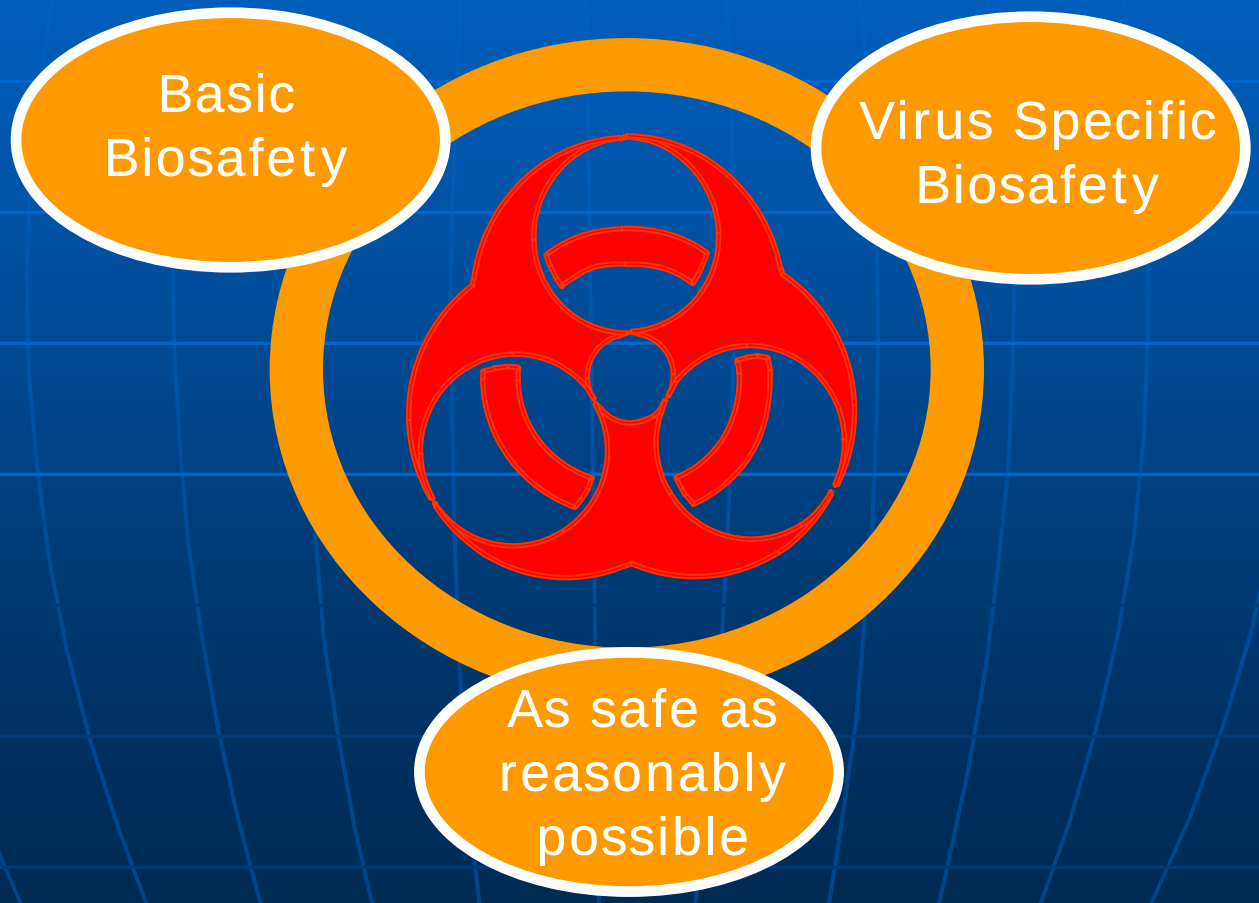
Flaviruses (Togaviruses) - Group B Arboviruses
- –Tick-borne encephalitis virus

Herpesviruses (alpha)
- –Herpesvirus simiae (Herpes B or Monkey B virus)

Paramyxoviruses
- –Equine morbillivirus

Hemorrhagic fever agents and viruses as yet undefined

The Review Should Follow Biology and Common Sense



IBC Forms and Responses

Calvin tries filling out an IBC form...

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...and working on the IBC

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IBC Forms and Responses

- The form should ask the right questions
- The form should provide some guidance, but not specific answers
- What should the IBC do when the PI doesn't understand the problem?
- What about reagents/animals generated elsewhere (commercial and noncommercial sources)? Custom produced viral vector stocks and "home grown" vector systems
  - Quality Control Issues

What the IBC Needs to Know

- What viral vectors will be used?
- What experiments will be done with recombinant DNA and/or viral vectors?
- 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?
- Reagent cycle: “Cradle to grave”

IBC Forms and Responses

- How to handle “blanket” protocols that cover many kinds of vector systems and a myriad of expressed genes
- What won’t be done in the experiments can be as important as what will be done

Basic Biosafety Concerns

- What is the host range of the parental virus? (infection vs. replication)
- Has anything been done to extend the host range of the vector?
- What is the pathogenicity of the parental virus?
- Has anything been done to extend the pathogenicity? (oncogenes, toxin genes, etc.)
- Can the recombinant DNA be mobilized? (viral vs. nonviral DNAs...complementation vs. recombination)
- Is the free DNA/RNA infectious?

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?

Recombinant DNA and Viral Vectors

- What is a virus?
  - It's small and hard to manipulate…
- Viral life cycles and viral life styles
- Recombinant DNA applications that involve viral vectors:
  - Replication competent viral vectors
  - Replication defective viral vectors
  - Cells and animals with viral vectors
  - Expression of genes (cDNAs, miRNA, etc)

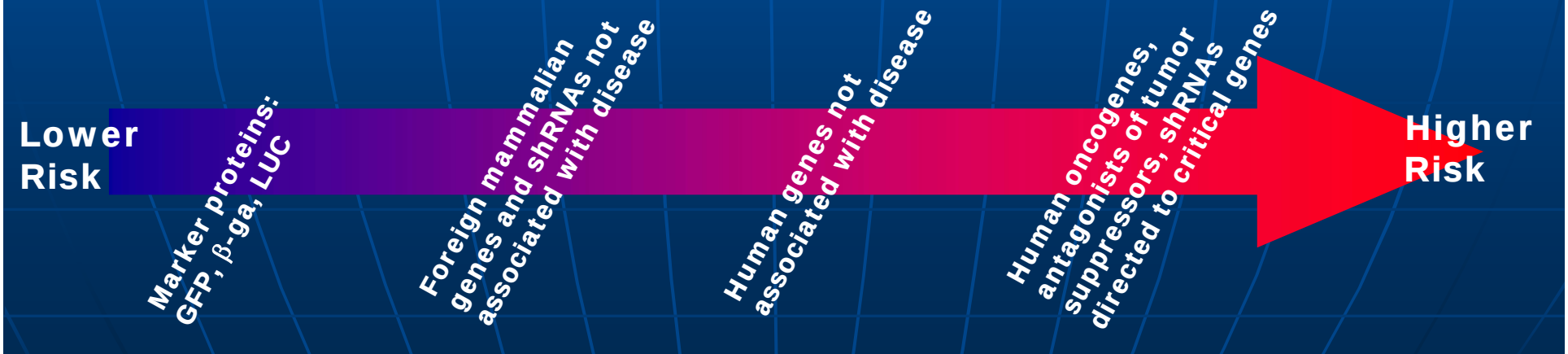
Things Viruses Do…

Different viral vectors do things differently

- Modify host genome
- Modify host immune response
- Remain latent
- Circulate in blood or remain in tissues
- Shed from the host
  - Bedding and excreta
  - Aerosol
- Pathogenic to host
- Recombine with other viruses (can happen during production or in vivo after introduction into the animal)

Developing a Broader Sense of Risk for rDNA and Viral Vectors

- Biological function of transgene
- Biological control
  - Permissive host (or permissive grafted host)
  - Immunity for viral vector
- Immunity evoked by transgene
  - Human genes
  - Regulatory RNAs



Comprehensive gene information: http://atlasgeneticsoncology.org/index.html

As Safe as Reasonably Possible

- Biological barriers are your best protection: If the vector won't replicate in a human…
- Physical barriers (BSCs, gloves, masks, clothing, 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

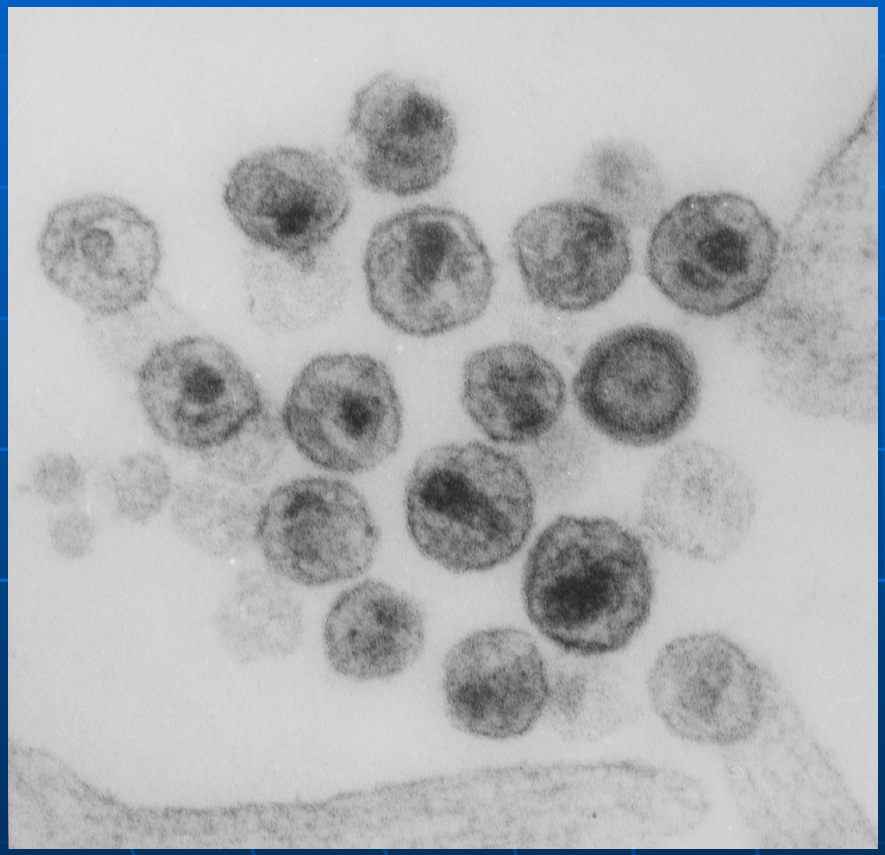
Quality Assurance

- Is the laboratory infrastructure capable of supporting the experiments
- Are the SOPs supplied with the IBC registration sufficient to cover the activities
- How current are the SOPs relative to the laboratory activities
  - IBC renewals and updates
- Monitoring laboratory activities

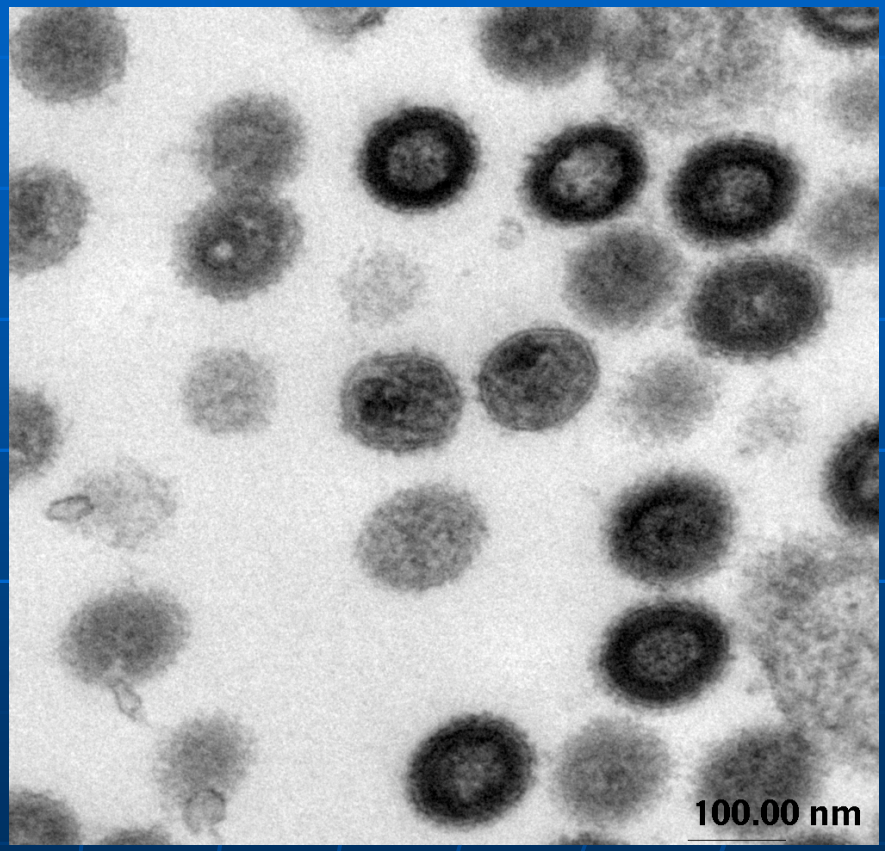
What's Being Done with the Vector?

- Types of manipulations
  - Culturing, titering, concentration, purification
- Introduction into animals
  - And will the viral vector be coming back out?
    - Cells and explants
    - Pathology samples
    - Wastes
- Does the nucleic acid of the viral vector present a risk

Lentiviral Vector Considerations



Wild Type HIV-1 Particles



Mutant HIV-1 Particles

Useful Guidance for Lentiviral Vectors

Biosafety Considerations for Research with Lentiviral Vectors

Recombinant DNA Advisory Committee (RAC) Guidance Document

Background: The use of lentiviral vectors has been increasing because the vector system has attractive features; however, such research also raises biosafety issues. The NIH Office of Biotechnology Activities has received frequent questions regarding the appropriate containment for lentiviral vectors, particularly those derived from HIV-1. Because the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (*NIH Guidelines*) do not explicitly address containment for research with lentiviral vectors, the RAC was asked to provide additional guidance for institutional biosafety committees (IBCs) and investigators on how to conduct a risk assessment for lentiviral vector research. At the March RAC 2006 meeting (webcast), the RAC offered the following findings and recommendations.

http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html

- The number of recombination events needed to reassemble the virus
- ***Is the entire virus present ?***
  - **env** (and tat) deletions common in lentiviral vectors

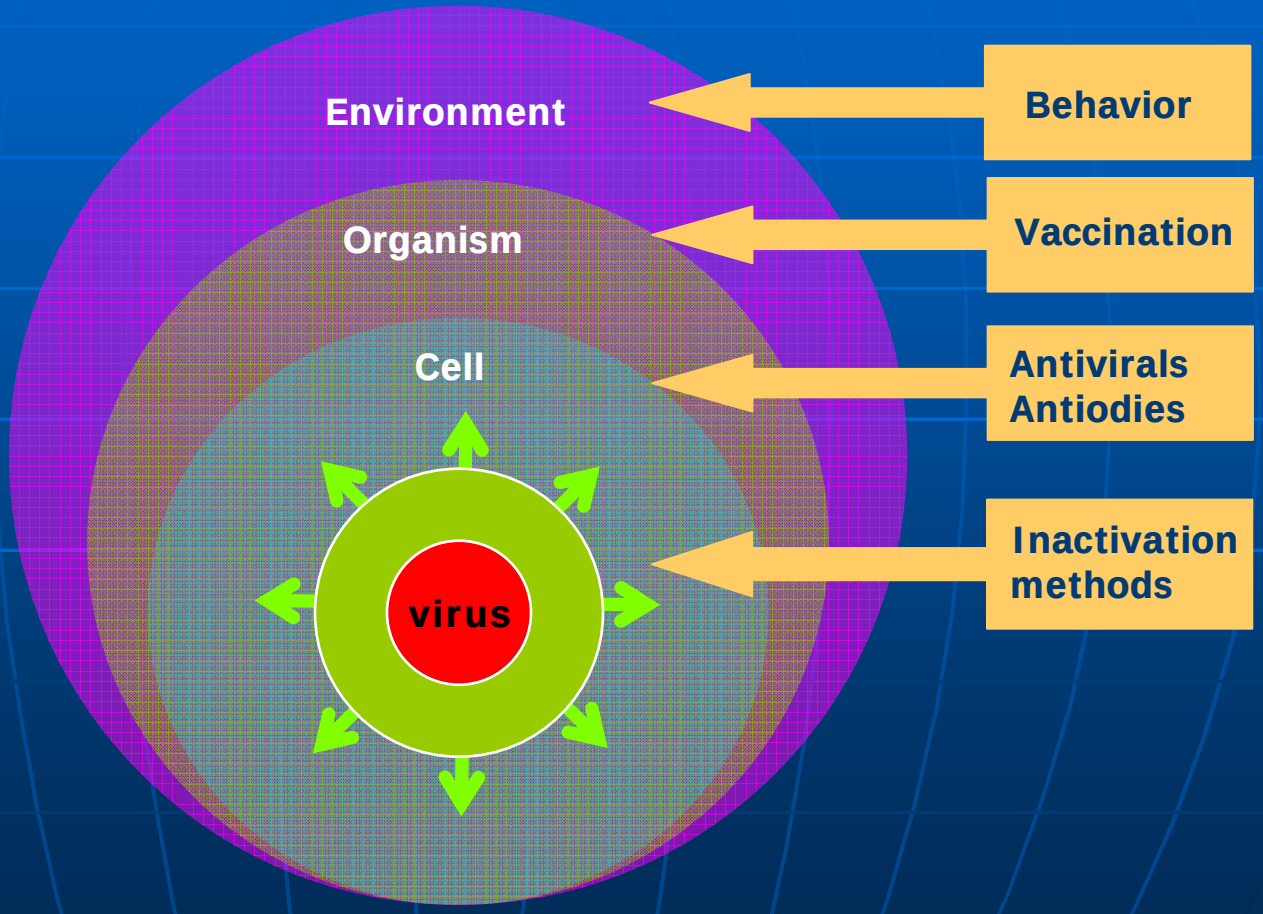
Lentiviral Vectors

- Env-deleted lentiviral vectors complimented by VSV-G do not appear to give rise to replicating viruses
- Lentiviral vectors do not successfully recombine with any known endogenous viruses
- Nature of transgene
- Integration/insertional mutagenesis
  - One of the few instances where antivirals can block
  - But there has to be advnaced planning
- Lentiviral vector infection of human cells can pose special risks
- In some cases, the literature that comes with commercial lentiviral vectors is misleading
- It is not easy to characterize a complex retroviral library (commercial or noncommercial)

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...

Mitigation Measures

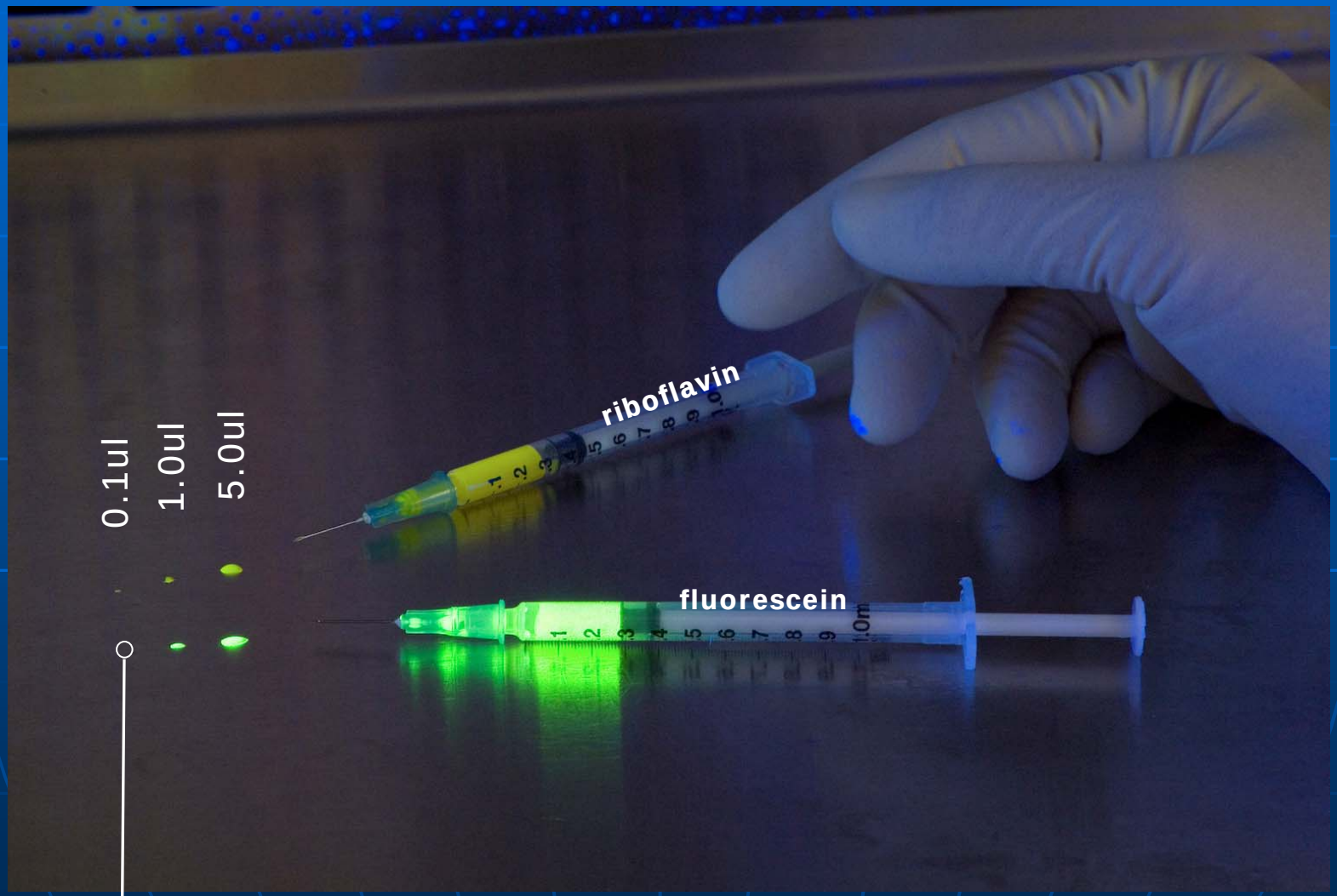


Process Controls and Training: Fluorescent Markers

- Fluorescent materials for tracking materials prior to use with agent/vector
- 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

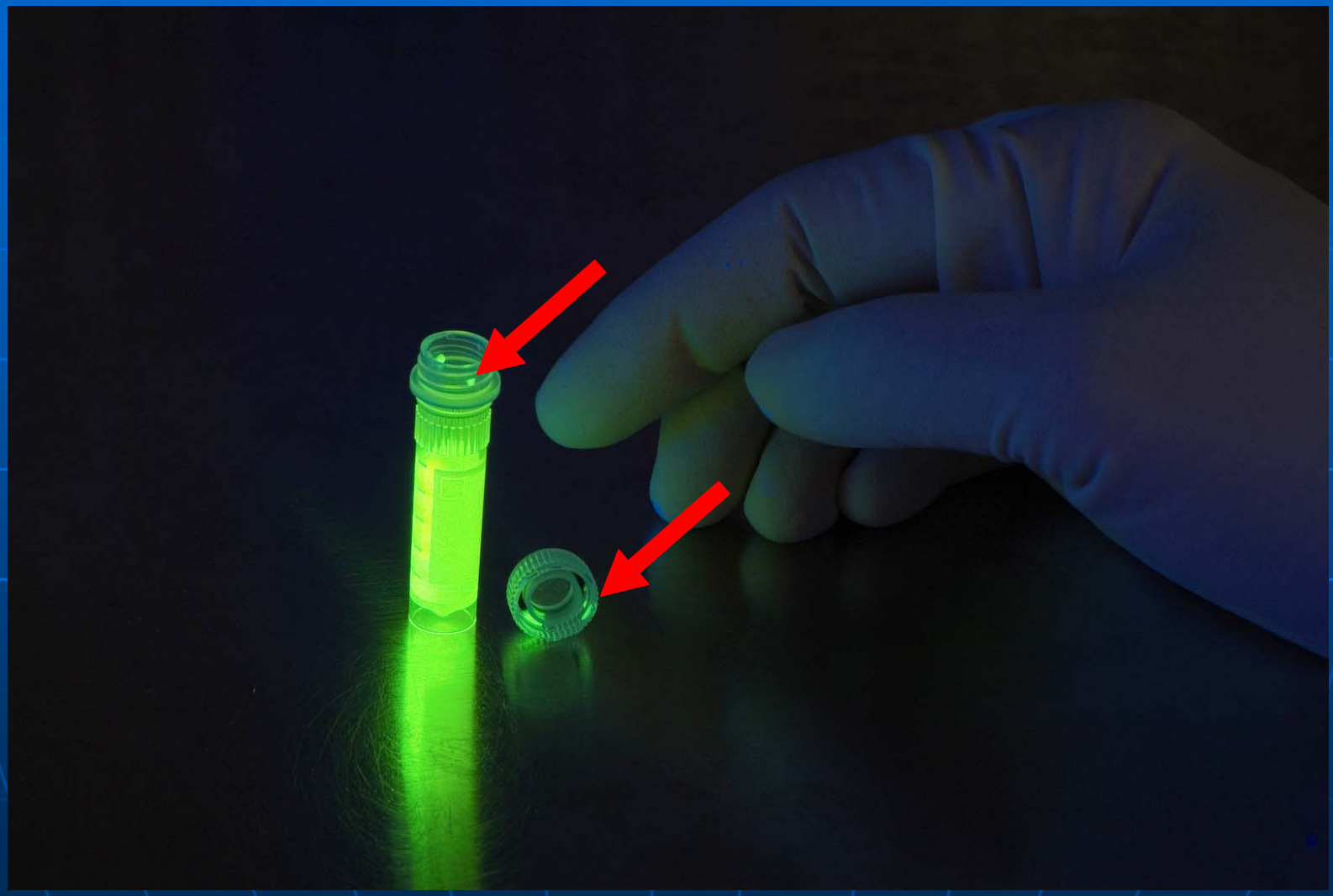
Process Controls & Training

Riboflavin and Fluorescein



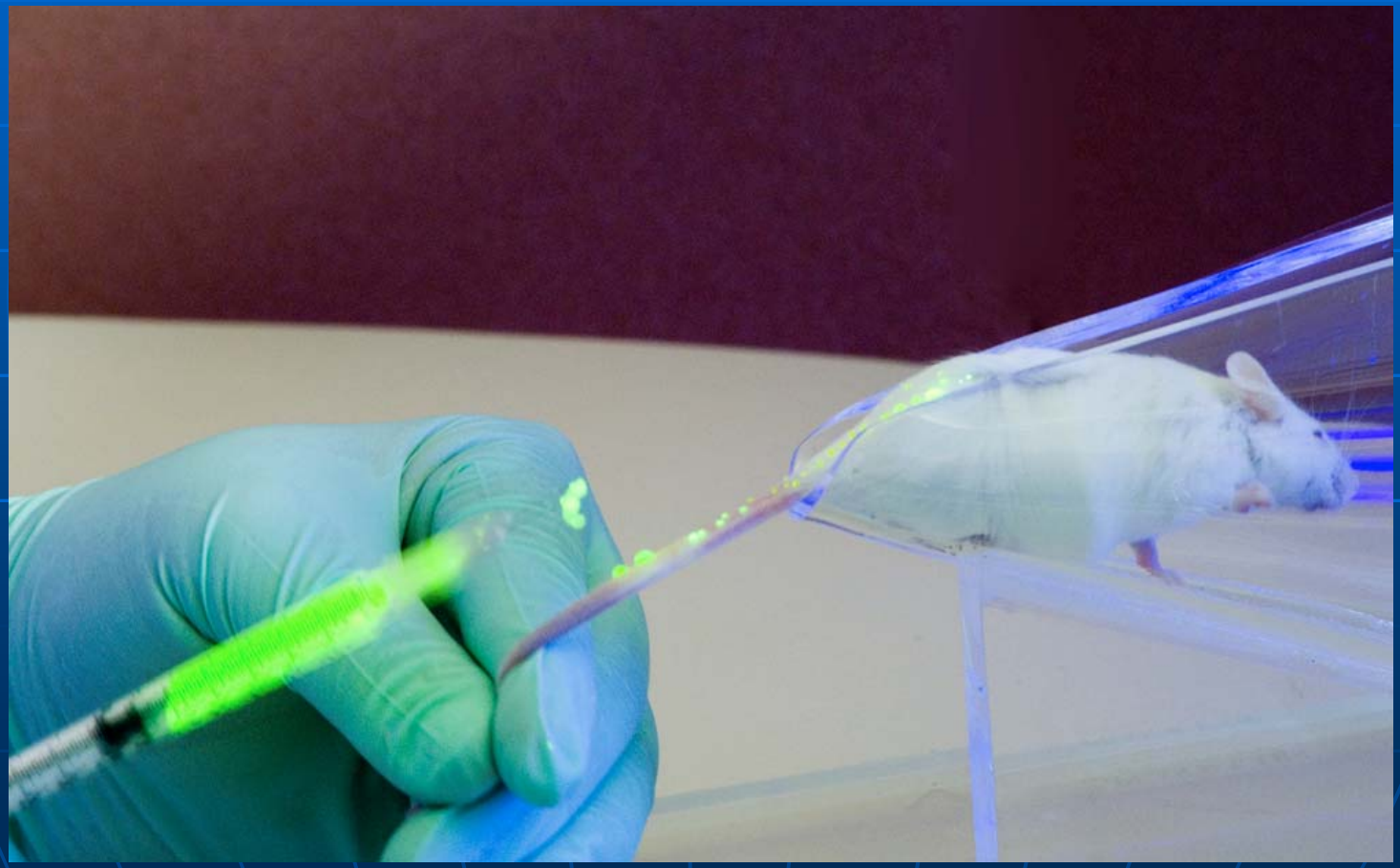
Fluorescein photobleaches after exposure to ultraviolet light

Material Retained on Threads of Cap



Transfer materials to a clean tube to eliminate contamination

Post-Injection Leakage



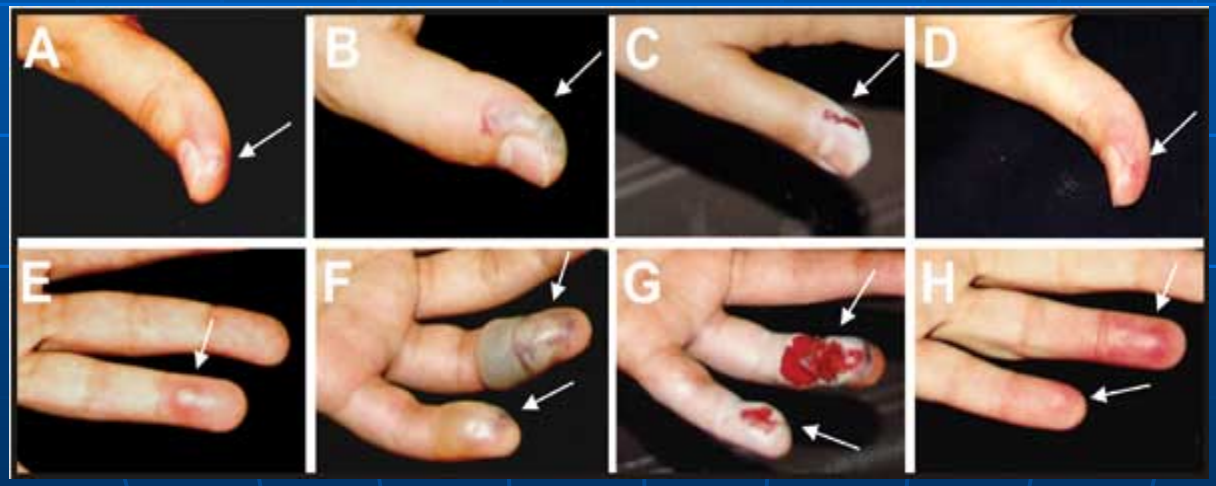
Where’s the Spill?

Engineering Controls and PPE

- Sharps !
- Do the physical barriers match the risk?
- Large scale considerations
- Is the PPE sufficient
  - Aerosols
  - Centrifugation
- Worst case scenarios
  - Spill drill

Sharps

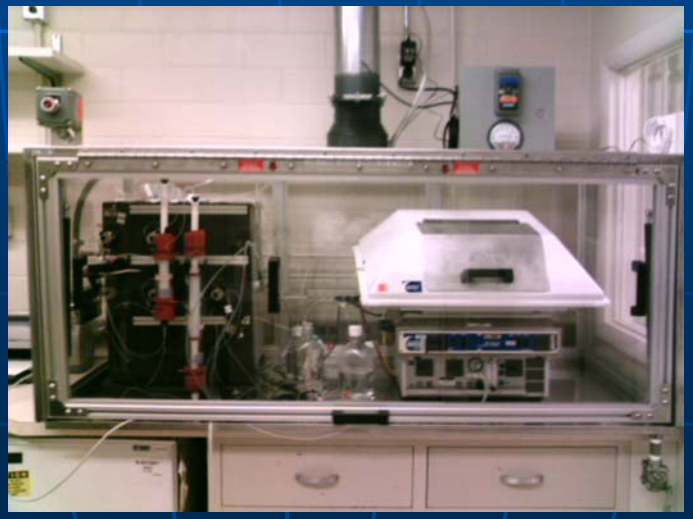
- In most cases, alternatives methods are available



Nissin et al., (2003) Emerging Infectious Diseases, Volume 9, Number 6, June 2003. Accidental Infection of Laboratory Worker with Vaccinia

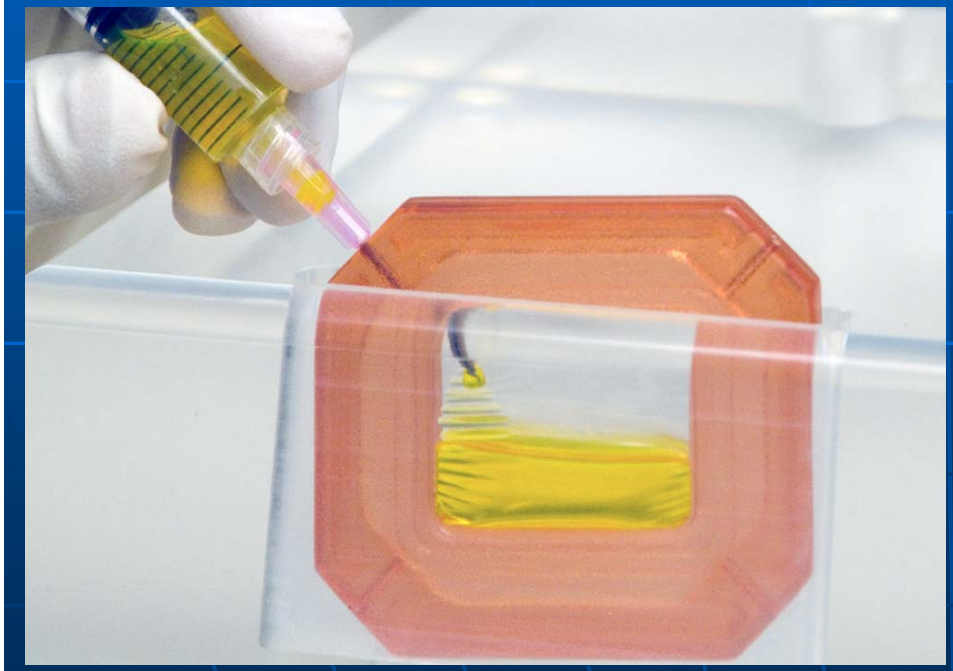
Mitigation of Risk

- Engineering Controls



Mitigation of Risk

- Engineering Controls cont'd
  - Needle pointed away from hands



Planning for the Worst

- Would practice with a fluorescent marker help?

1 liter of recombinant HSV vector

- Spill drills/spill clean-up kits
- Contingency Planning

Would Vaccination Help?

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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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0.5-cm vesicle was noted above the left canthus (Figure 1).
Left ocular range of motion, including palpebral motion,
was severe
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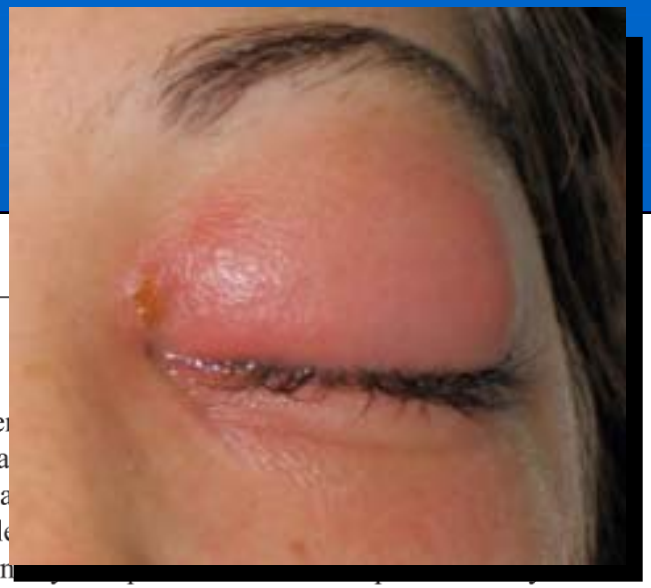


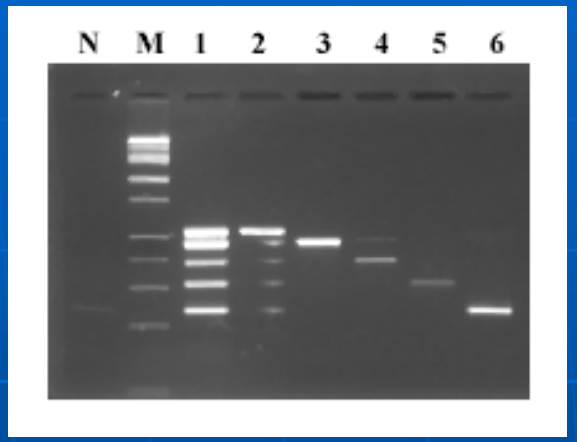
Quality Control: Are You Sure You Know What You Are Getting?

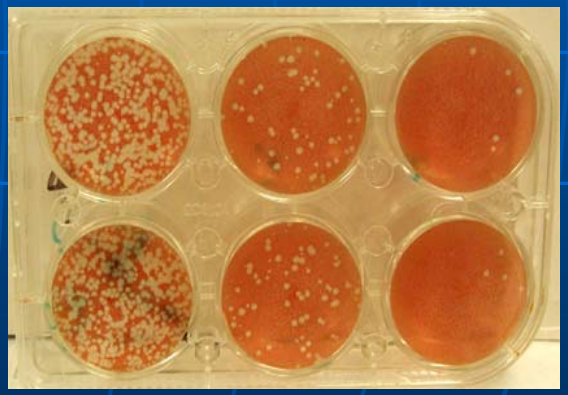
Virology Tool Kit

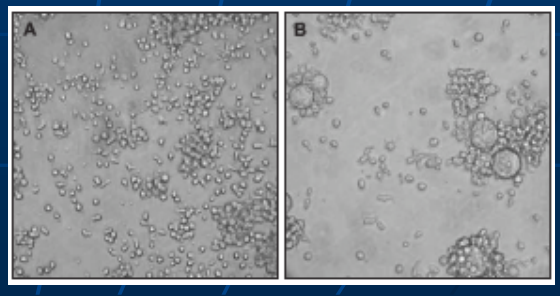
- Detecting and validating viruses/vectors
  - Cytopathic effects
  - Transformation
  - Molecular assays (ELISA, antibodies, PCR etc.)
  - **When in doubt *sequence* the vector**

Useful Ways to Monitor for Viral Vector Quality

- PCR/Sequence
- Plaque/Replication Assays
- What to monitor
  - Viral vector stocks
  - Producer cells
  - Transduced/carrier cells
- What to monitor for:
  - RCA
  - Endogenous contaminants







Experiments Involving Animals

- Interaction between the IBC and ACUC committees is needed for comprehensive safety program
- Co-mingled memberships between the two committees leads to consistency in the review process
- IBC and ACUC forms with complementary questions results in thorough review

Acknowledgements

- Steve Hughes
- Joe Kozlovac
- Alan Kane
- Jonathan Summers
- Julie Bullock
- Misty Hawes

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