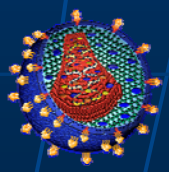
# Using Viral Vectors in Animal Research

**Stephen Hughes, PhD**
**Director, HIV-Drug Resistance Program**
**NCI-Frederick**



# Basic Biosafety Concerns

- Can the recombinant DNA be mobilized? (viral vs. nonviral DNAs...complementation vs. recombination)
- Is the free DNA (or RNA) infectious?
- 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.)

# As Safe as Reasonably Possible

- Biological barriers are your best protection: If the vector won't replicate in a human……
- Physical barriers (hoods, 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 or PEP can help in some cases)
- Know what you are working with: Quality control for cells, animals and vectors.

# Who Are We Protecting?

- Care takers/animal husbandry personnel
- Research laboratory staff
  - 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
- IBC and the ACUC

# How Is an Animal Different from a Petri Dish?

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

# Cultured Cells Don’t Sneeze

# Cultured Cells Don’t Bite

# 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
- Viral DNA is not (normally) integrated: Poxvirus, adenovirus, herpesvirus, rhabdovirus, alphavirus

# Host Range, Replication and Pathogenicity of Viral Vectors

- What ifs the pathogenicity of the parental virus?
- What are the routes of infection (aerosols)?
- What is the host range of the parental virus (replication)?
- Can the virus infect hosts where it will not replicate?
- Has anything been done to change the host range?
- Has anything been to change the pathogenicity?

# Special Considerations for Retroviral/Lentiviral 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 immunosuppressed 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

# Primates Can Get Tumors from an MLV-based Vector

JOURNAL OF VIROLOGY, July 1994, p. 4241–4250
0022-538X/94/$04.00+0

Vol. 68, No. 7

## Characterization of Replication-Competent Retroviruses from Nonhuman Primates with Virus-Induced T-Cell Lymphomas and Observations Regarding the Mechanism of Oncogenesis

ELIO F. VANIN,[1]* MICHELE KALOSS,[1] CHRISTINE BROSCIUS,[1] AND ARTHUR W. NIENHUIS[2]†

*Genetic Therapy Inc., Gaithersburg, Maryland 20878,[1] and Clinical Hematology Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20892[2]*

Received 2 February 1994/Accepted 28 March 1994

**Rapidly progressive T-cell lymphomas were observed in 3 of 10 [illegible] autologous transplantation of enriched bone marrow [illegible]**

# Immunosuppressed Human Patients Can Get Tumors from an MLV-based Vector

RESEARCH ARTICLE

## *LMO2*-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hacein-Bey-Abina,[1,2*] C. Von Kalle,[6,7,8] M. Schmidt,[6,7] M. P. McCormack,[9] N. Wulffraat,[10] P. Leboulch,[11] A. Lim,[12] C. S. Osborne,[13] R. Pawliuk,[11] E. Morillon,[2] R. Sorensen,[19] A. Forster,[9] P. Fraser,[13] J. I. Cohen,[15] G. de Saint Basile,[1] I. Alexander,[16] U. Wintergerst,[17] T. Frebourg,[18] A. Aurias,[19] D. Stoppa-Lyonnet,[20] S. Romana,[3] I. Radford-Weiss,[3] F. Gross,[2] F. Valensi,[4] E. Delabesse,[4] E. Macintyre,[4] F. Sigaux,[20] J. Soulier,[21] L. E. Leiva,[14] M. Wissler,[6,7] C. Prinz,[6,7] T. H. Rabbitts,[9] F. Le Deist,[1] A. Fischer,[1,5†‡] M. Cavazzana-Calvo[1,2†]

We have previously shown correction of X-linked severe combined immunodefi-
ciency [SCID-X1, also known as γ chain (γc) deficiency] in 9 out

long terminal repeat (LTR) driven MFG vector (*4*) resulted in the development of a functional adaptive immune system (Fig. 1A) (*2*). The clinical benefit has been so far sustained for more than 4 years in the first two treated patients; potentially, this sustained efficacy could be explained in part by the transduction of pluripotent progenitors with self-renewal capacity (*5*, *6*). The main potential risk of retrovirus-mediated gene transfer is insertional mutagenesis resulting from random retroviral integration. This could either activate proto-oncogenes over long distances (up to 100 kbp) or inactivate tumor-suppressor genes, ultimately leading to malignancies. To date, this risk has been considered very low, because it has never been observed in a clinical trial. Furthermore, only recently has evidence become available that insertion of replication-defective retrovirus vectors could contribute to malig

# Human Cells Passed in Nude Mice Can Acquire Murine Retroviruses…

Cancer Res. 1989 Feb 1;49(3):625-8. Related Articles, Links

**Mouse retroviral sequences acquired by cell lines after passaging through nude mice detected by hybridization of the fms probe pSM3.**

Walker C, Nettesheim P, Barrett JC, Jirik FR, Sorge J, Joyce M, Gilmer T.

National Institute of Environmental Health Sciences, Laboratory of Pulmonary Pathobiology, Research Triangle Park, North Carolina 27709.

The expression of a large RNA tran

# 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.

# Retroviral Recombination Does Not Require Homology

1: Science. 1993 Jan 8;259(5092):234-8. Related Articles, Links

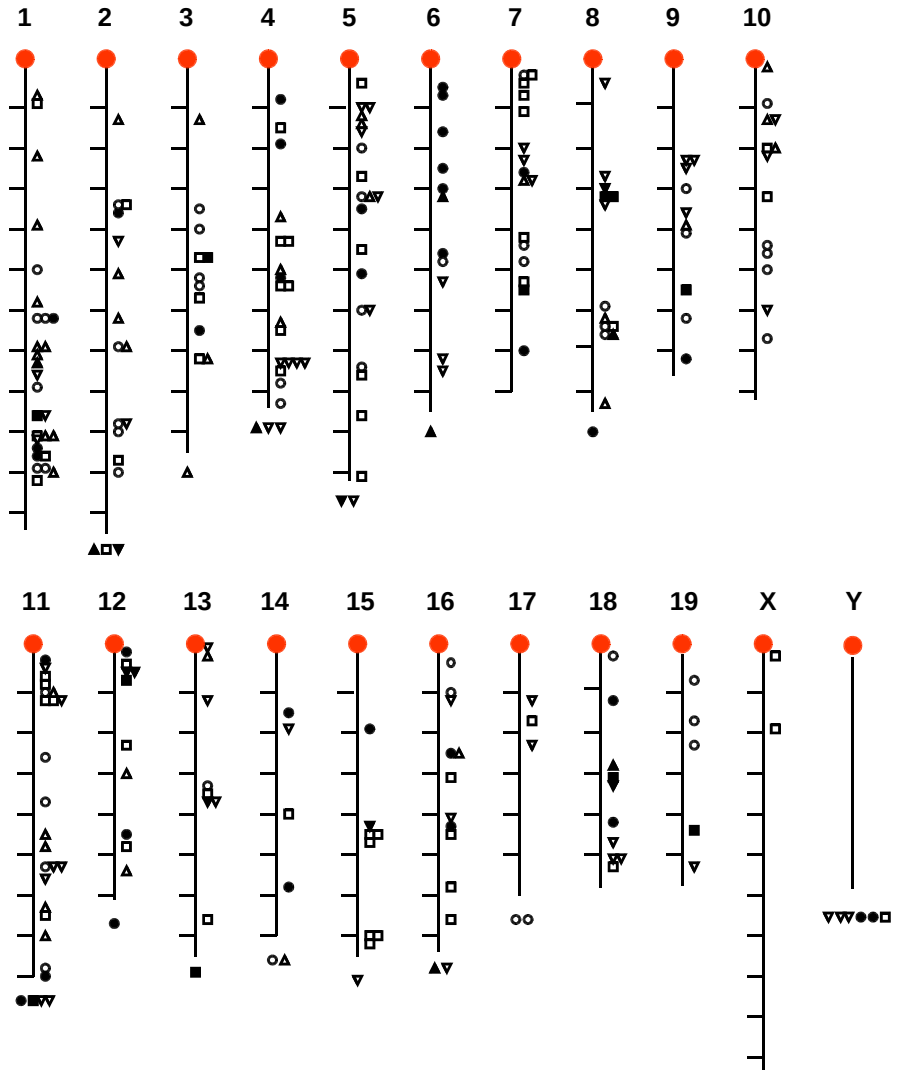
**Rate and mechanism of nonhomologous recombination during a single cycle of retroviral replication.**

**Zhang J, Temin HM.**

McArdle Laboratory for Cancer Research, University of Wisconsin-Madison 53706.

Oncogenes discovered in retroviruses such as Rous sarcoma virus were generated by transduction of cellular proto-oncogenes into the viral genome. Several different kinds of junctions between the viral and proto-oncogene sequences have been found in different viruses. A system of retrovirus vectors and a protocol that mimicked this transduction during a single cycle of retrovirus replication was developed. The transduction involved the formation of a chimeric viral-cellular RNA, strand switching of the reverse t

# Endogenous Mouse Viruses




= 10 cM

= centromere

= EcoMlv

= PolyMlv

= MxPolyMlv

= XeroPolyMlv

= Mmtv

= Pltr

= Mltr

= Xltr

# Special Considerations for 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
- 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)

# 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: $10^{12}$

# Adenovirus Recombination

JOURNAL OF VIROLOGY, June 2004, p. 6200–6208 Vol. 78, No. 12
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.12.6200–6208.2004


## Common Structure of Rare Replication-Deficient E1-Positive Particles in Adenoviral Vector Batches

Pete Murakami,[1] Menzo Havenga,[2] Farah Fawaz,[1] Ronald Vogels,[2] Giuseppe Marzio,[2] Erno Pungor,[1] Jim Files,[1] Linh Do,[1] Jaap Goudsmit,[2,3] and Michael McCaman[1]*

*Process Development Department, Berlex Biosciences, Richmond, California,[1] and Crucell Holland BV, 2301CA Leiden,[2] and Center for Poverty-Related Communicable Diseases, Academic Medical Center, University of Amsterdam, Amsterdam,[3] The Netherlands*

Received 7 November 2003/Accepted 10 February 2004

**The use of the PER.C6 adenovirus packaging cell line in combination with a designated vector plasmid system, whereby the cell line and vector with E1 deleted have no sequence overlap, eliminates the generation of replication-competent adenovirus during vector production. However, we have found cytopathic effect (CPE)-inducing particles in 2 out of more than 40 large-scale manufacturing lots produced in PER.C6 cells. The CPE inducer was detected at a frequency of 1 event in 7.5 × $10^{12}$ vector particles. Despite amplification, it was not readily purified, indicating that the agent itself is replication deficient and requires the parental recombinant adenovirus serotype 5 (rAd5) vector for replication and packaging. Therefore, we designated the agent as a helper-dependent E1-positive region containing viral partic**
**ular structure of the HDEP genome, revealing an Ad**
**flanked by inverted terminal repeat**

# 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

# HepC in Vaccinia

PO Box 2345, Beijing 100023, China World J Gastroenterol 2004;10(18):2670-2674
Fax: +86-10-85381893 World Journal of Gastroenterology
E-mail: wjg@wjgnet.com www.wjgnet.com Copyright © 2004 by The WJG Press ISSN 1007-9327

• VIRAL HEPATITIS •

## A vaccinia replication system for producing recombinant hepatitisC virus

Ying-Song Wu, Yu Feng, Wen-Qi Dong, Yan-Ming Zhang, Ming Li
**Ying-Song Wu, Yu Feng, Wen-Qi Dong, Yan-Ming Zhang, Ming Li**, Institute of Tropical Medicine, First Military Medical University, Guangzhou 510515, Guangdong Province, China
**Supported by** the "863" Program of China, No.2001AA215171
**Correspondence to:** Dr. Ming Li, Institute of Tropical Medicine, First Military Medical University, Guangzhou 510515, Guangdong Province, China. mingli@fimmu.com

# Preventing/Controlling Viral Infections

- Biological barriers (virus won't infect and/or replicate in humans)
- Physical barriers (hoods, clothing, masks, etc.)
- Vaccination
- Antiviral therapy (post exposure)

# Physical Protection Should Match the Risk: What is the Expected Route of Infection?

# Avian Flu: Bad Ideas

- Each of the next few slides shows one (or more) obvious mistakes...
- Unfortunately these are NOT isolated examples...

# What is Wrong Here?

# What's Wrong Here?

# What's Wrong Here?

# 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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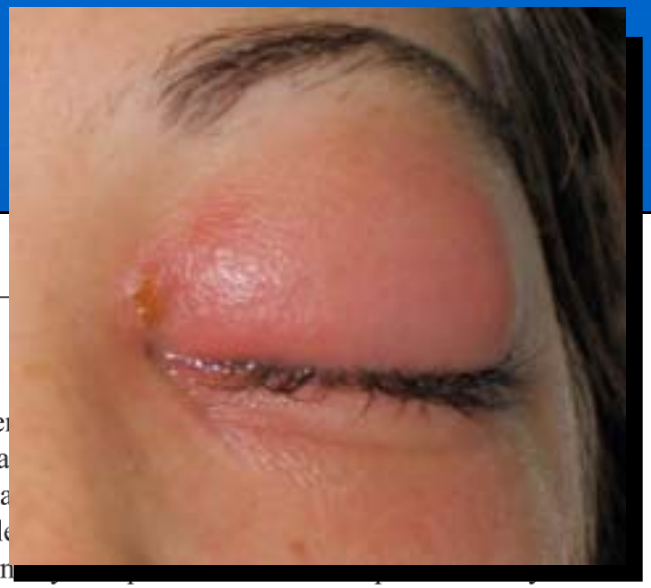
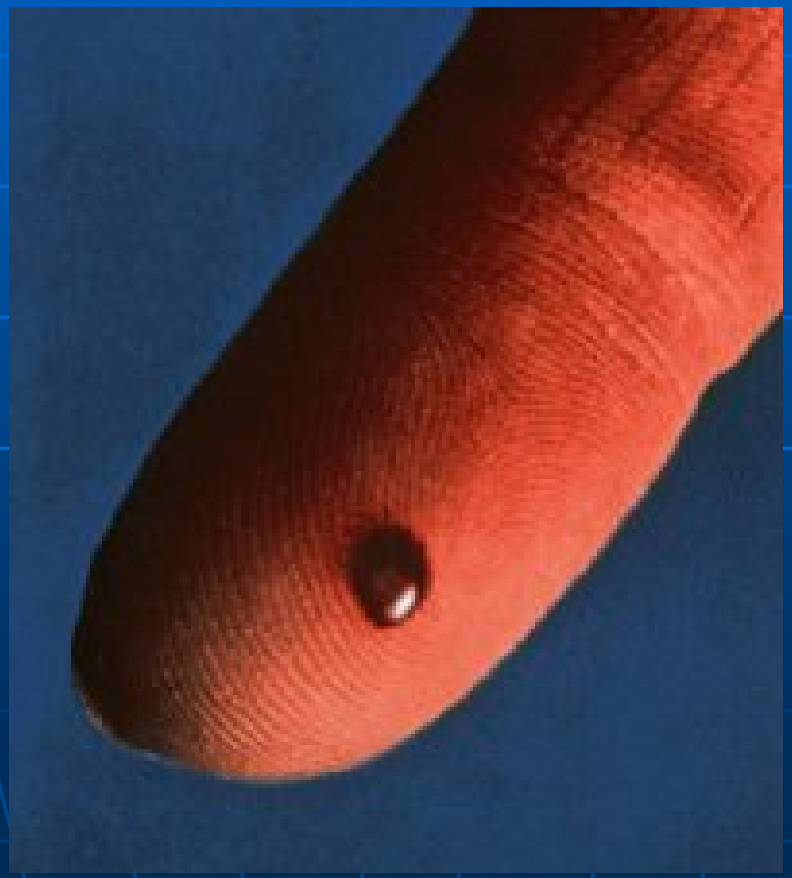


# Was She Wearing Eye Protection?

# Of Course Not……

# Needle Stick



# Vaccinia Virus Infection


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

# Antiviral Therapy

- There are no effective antiviral therapies for most viruses.
- Develop a post-exposure plan before the need arises:
  - The issues for intervention are often very complex
  - Timing is important
- There are effective anti-HIV-1 drugs, but these must be administered rapidly after an exposure (hours).
- Anti HIV-1 drugs can be used to block infections with HIV-1 based vectors, but the relative risks from the drugs and the vector must be weighed carefully and quickly.

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

# 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:
  - Endogenous/exogenous contaminants
  - Structure of the vector/nature of the insert
  - Replication competence

# Developing a Safe Procedure

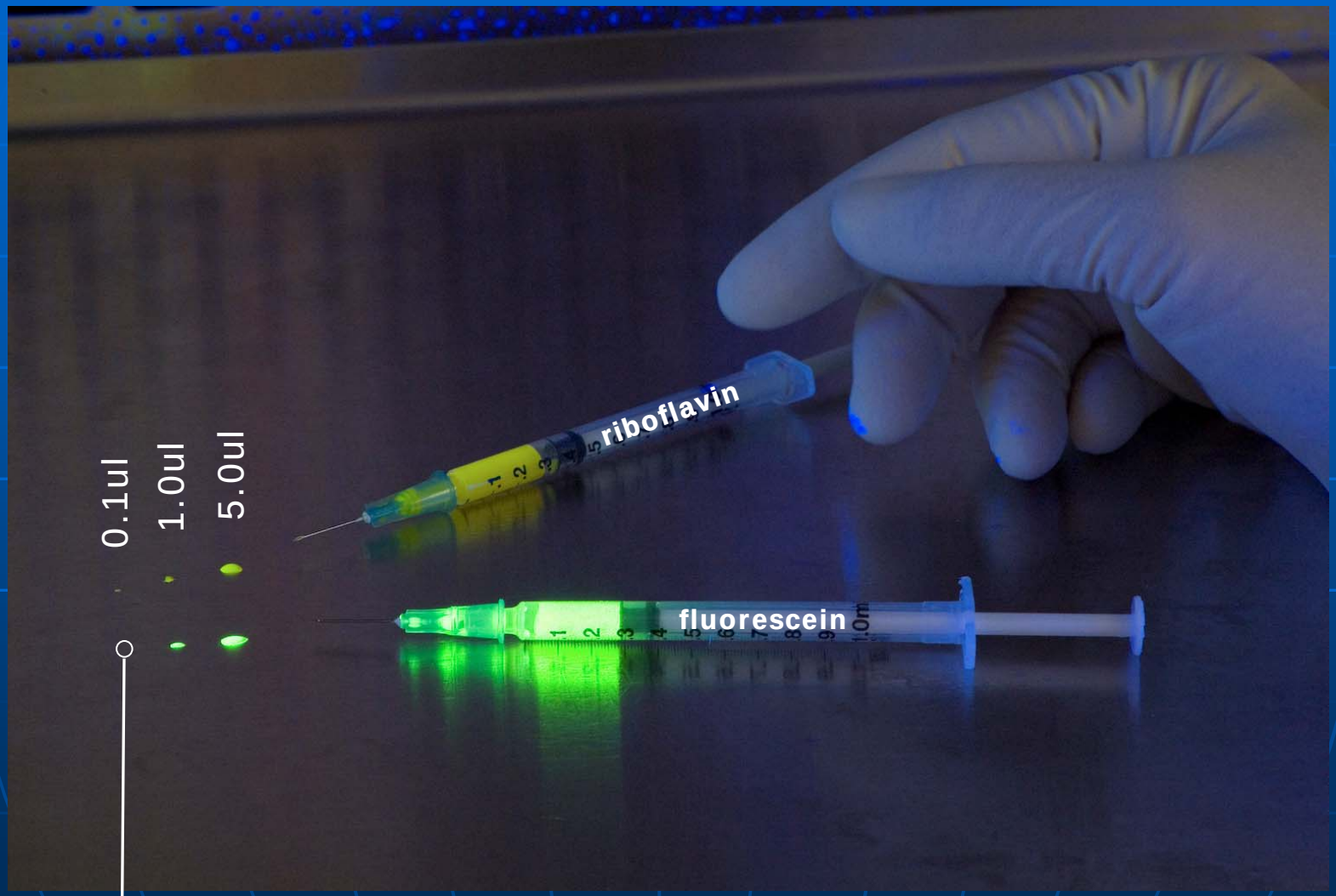
- Develop safe procedures before starting to work with viral vectors
- Make sure all the personnel know the risks
- Practice with safe reagents
- Make sure any contaminated material is disinfected

# Why We Like to 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

# Fluorescein Tracking of Spills

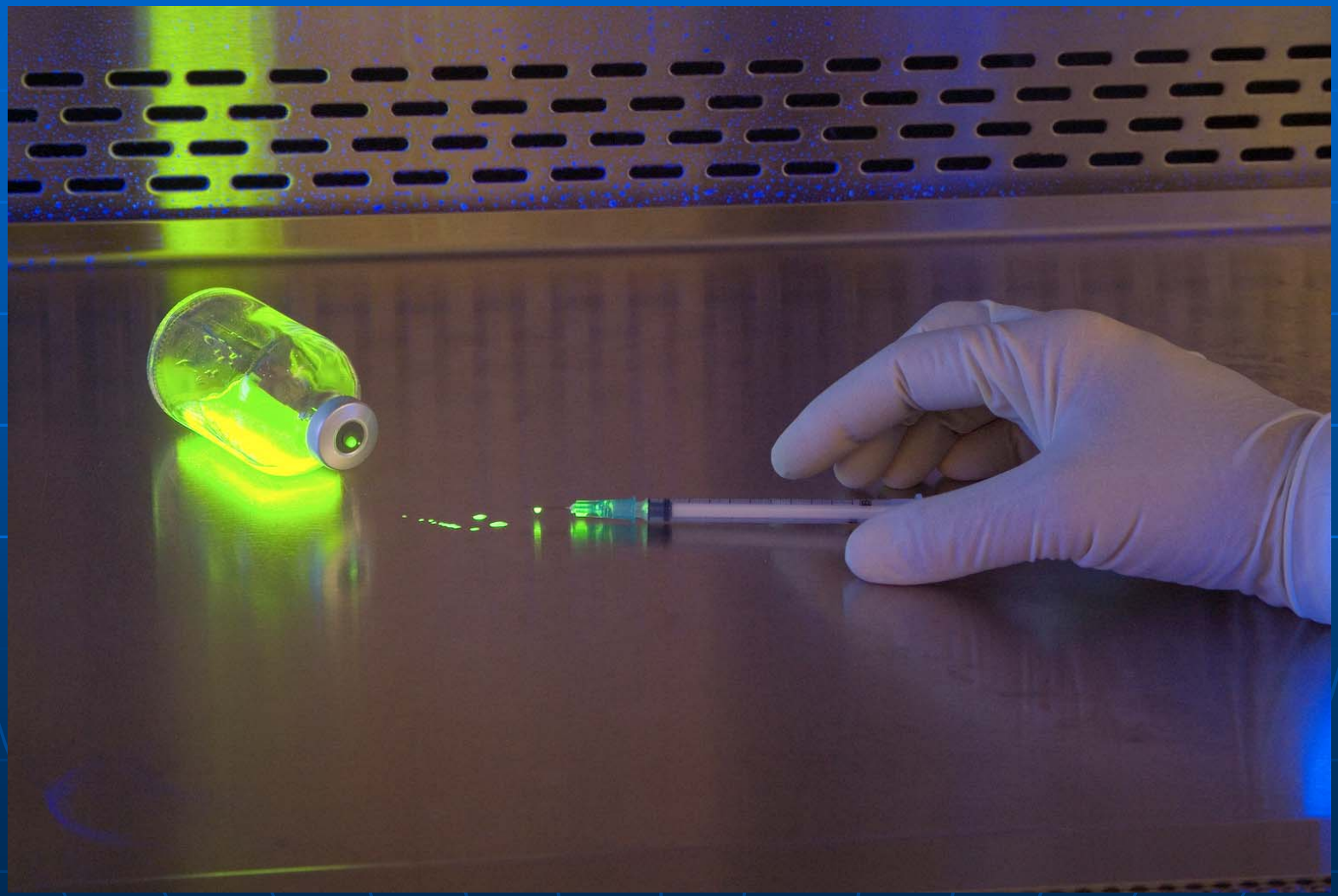
# Riboflavin and Fluorescein



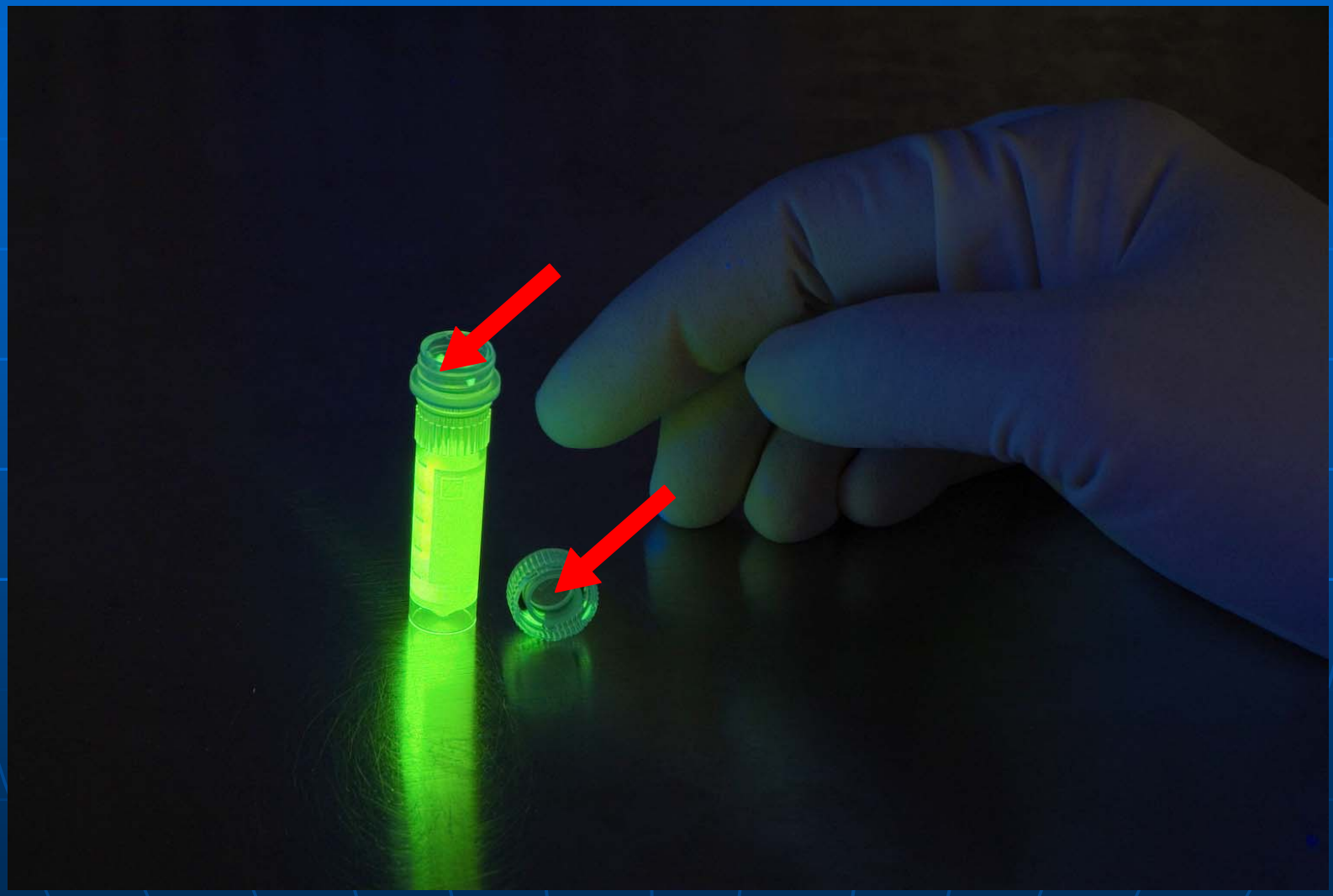

Fluorescein photobleaches after exposure to ultraviolet light

# Contents Under Pressure???

# Spray from Needle/syringe

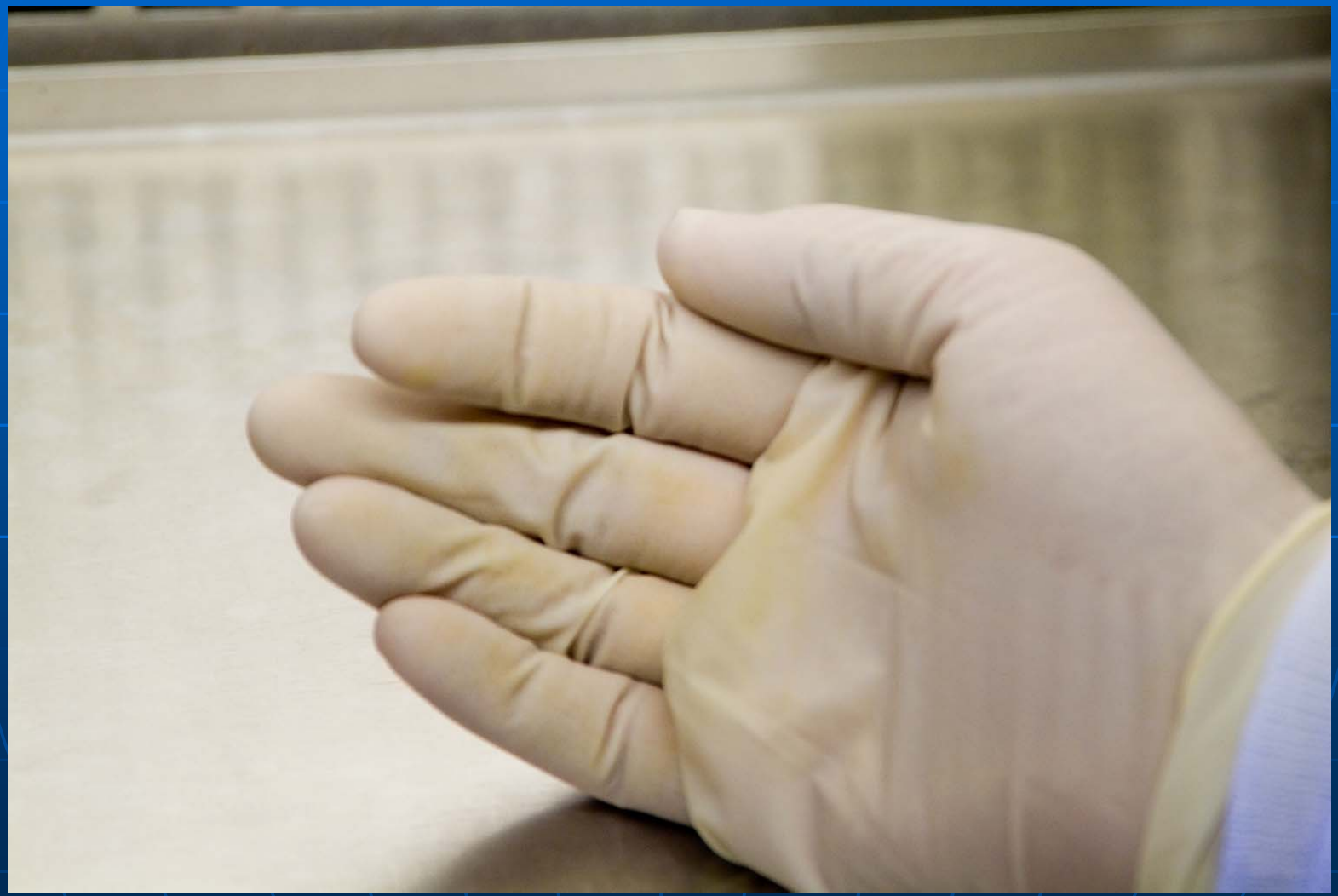


# Material Can Be Retained on the Cap

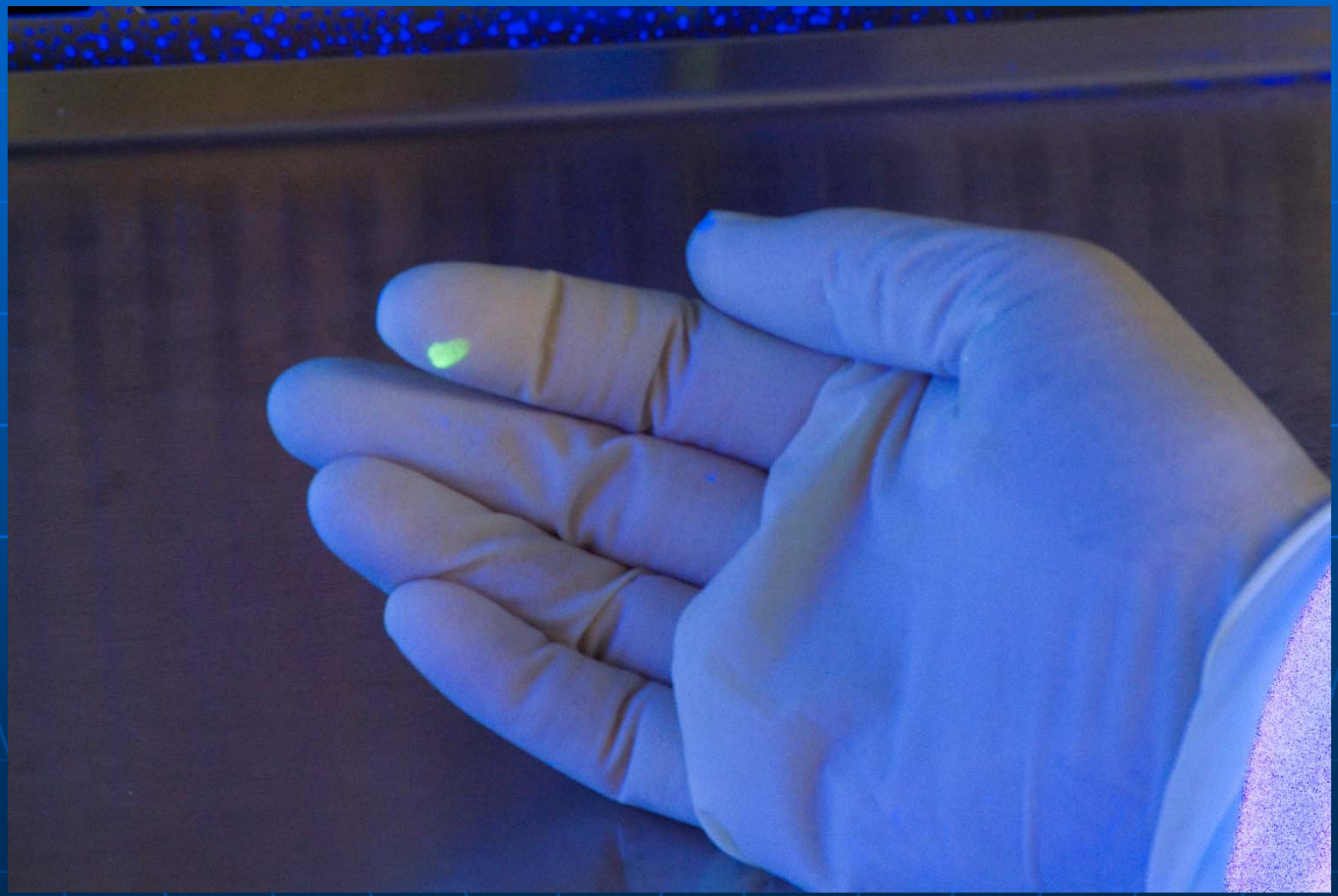


Transfer materials to a clean tube if the cap could be contaminated.

# Where’s the Spill?



# UV Light Exposure

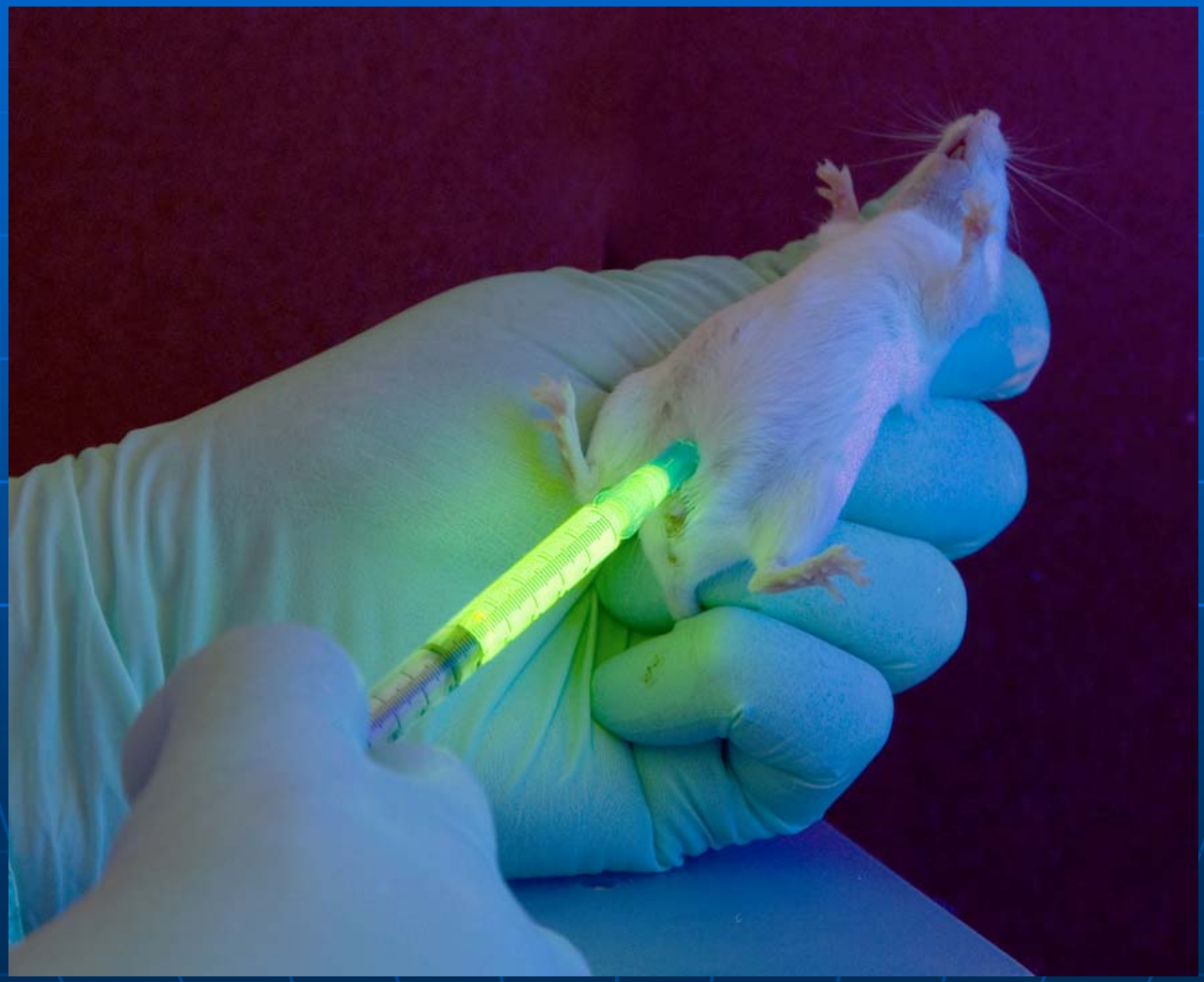


# Change Your Gloves

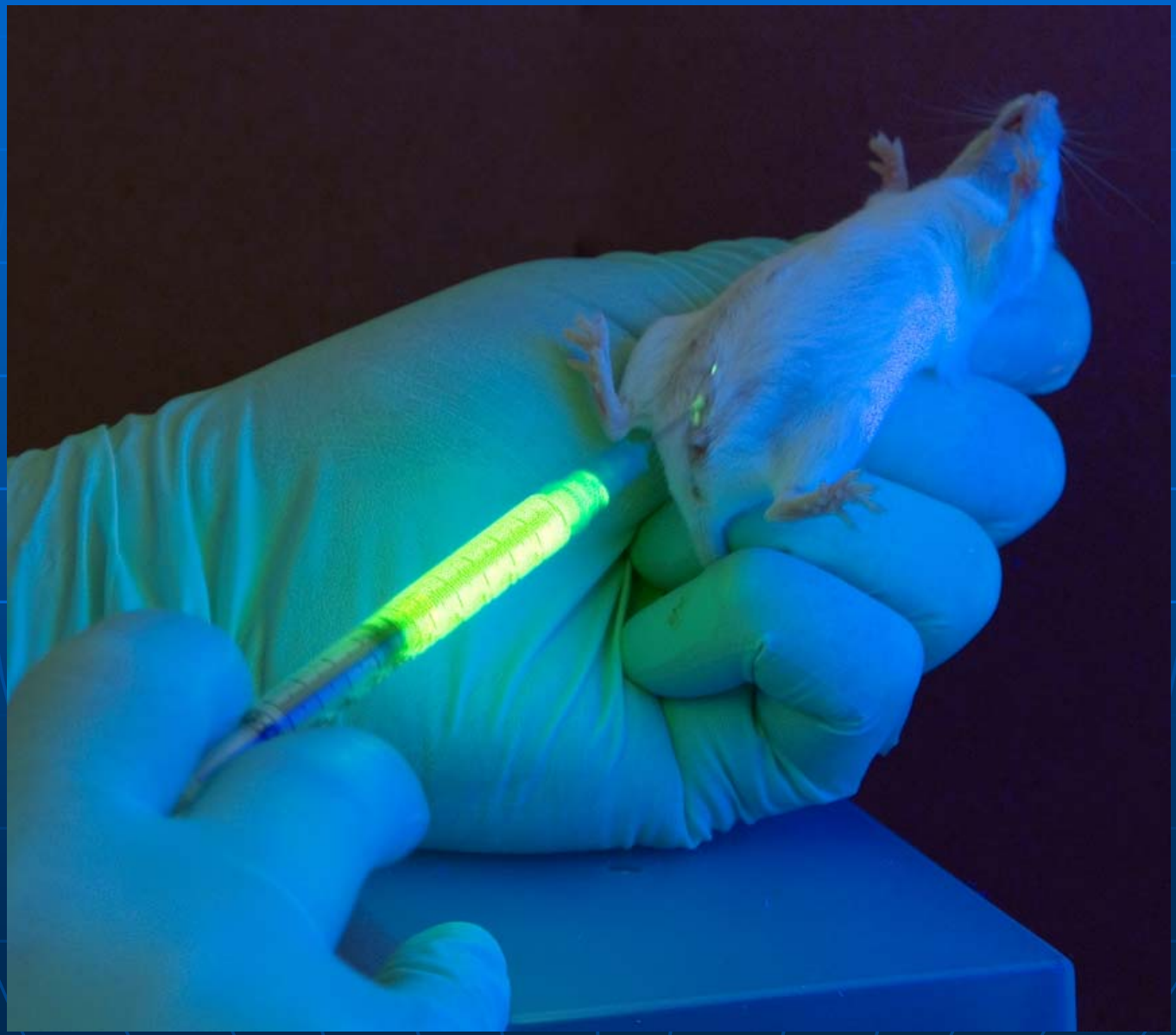
# Restraining, Injecting and Caging Mice

- Injecting virus into animals…inject the mouse….not yourself
- Using a restraint and appropriate injection technique
- Use appropriate caging

# IP Injection of Mice

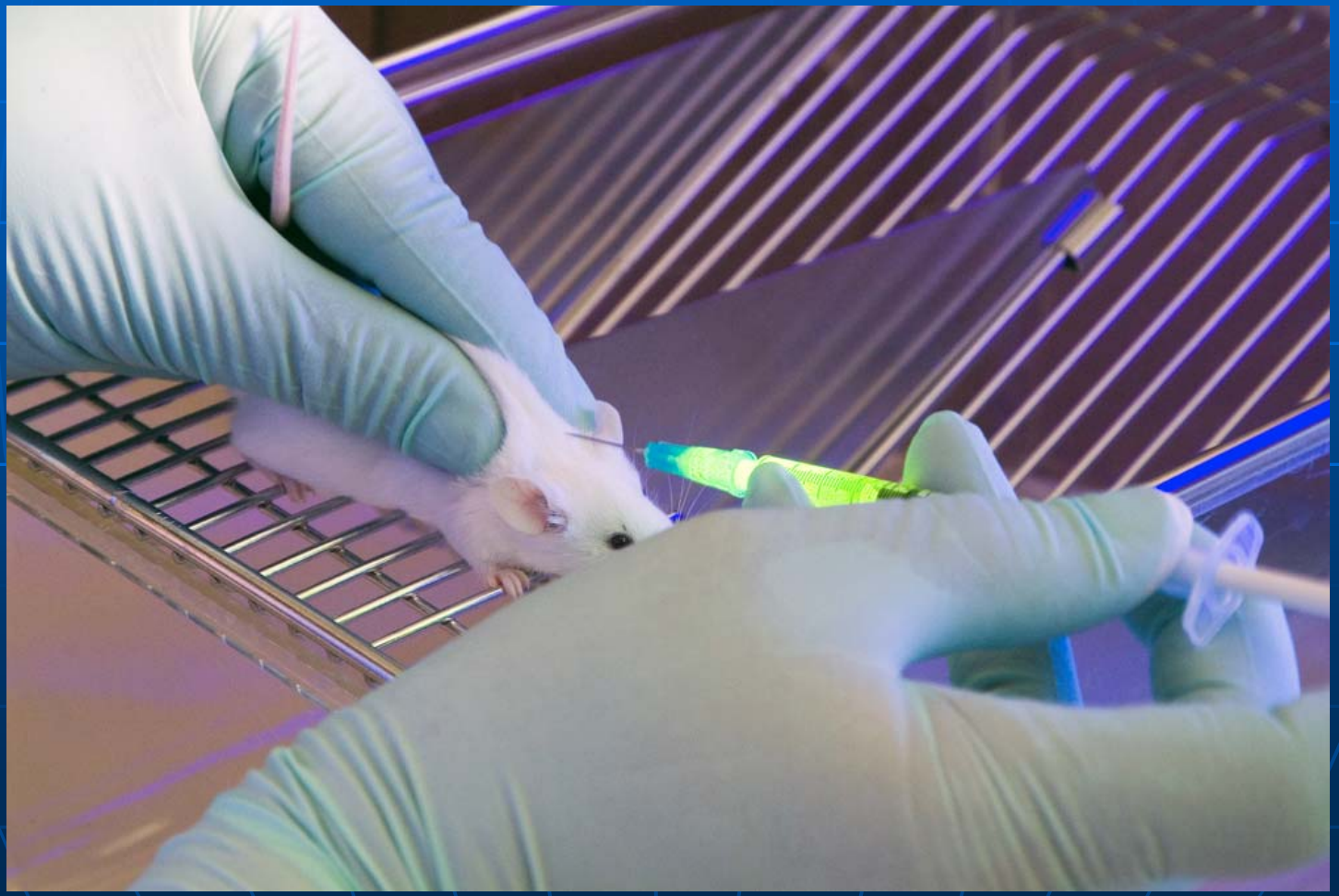


# Post-Injection Leakage



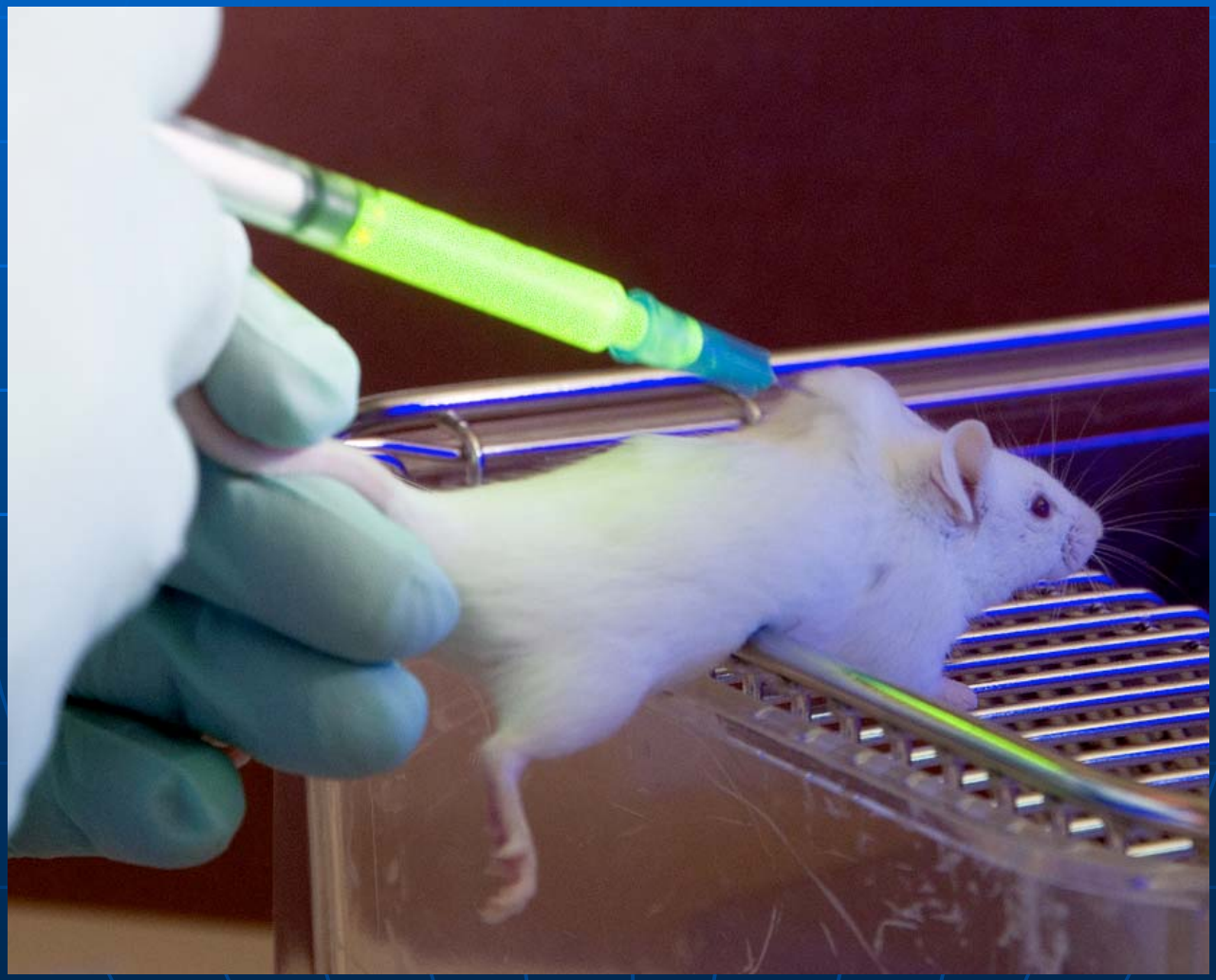
# SC Injection

- Hand more likely to have needle contact



# SC Injection of Mice

- When possible, position animal such that the needle isn't in line with a hand

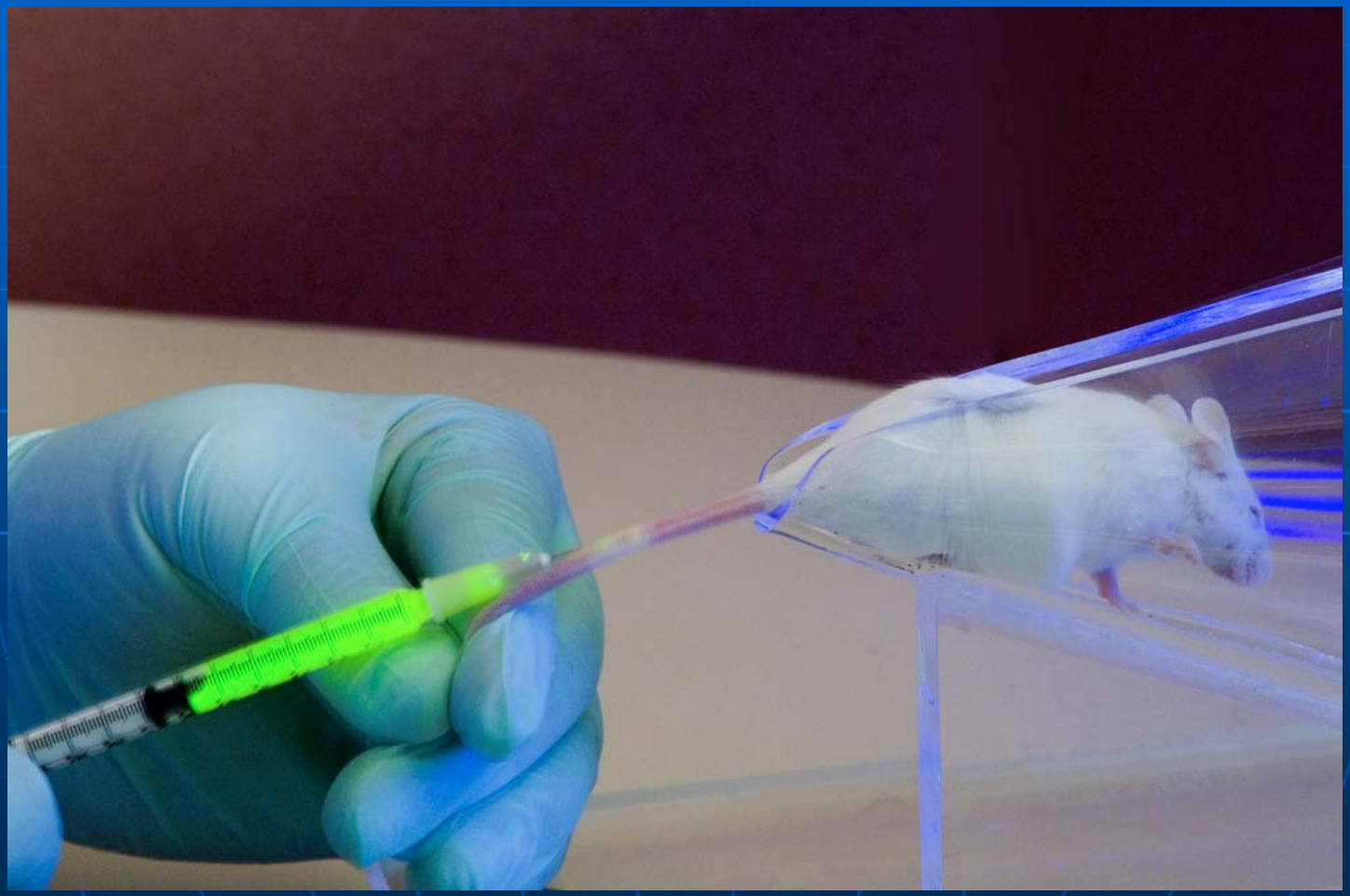


# Post-Injection Leakage

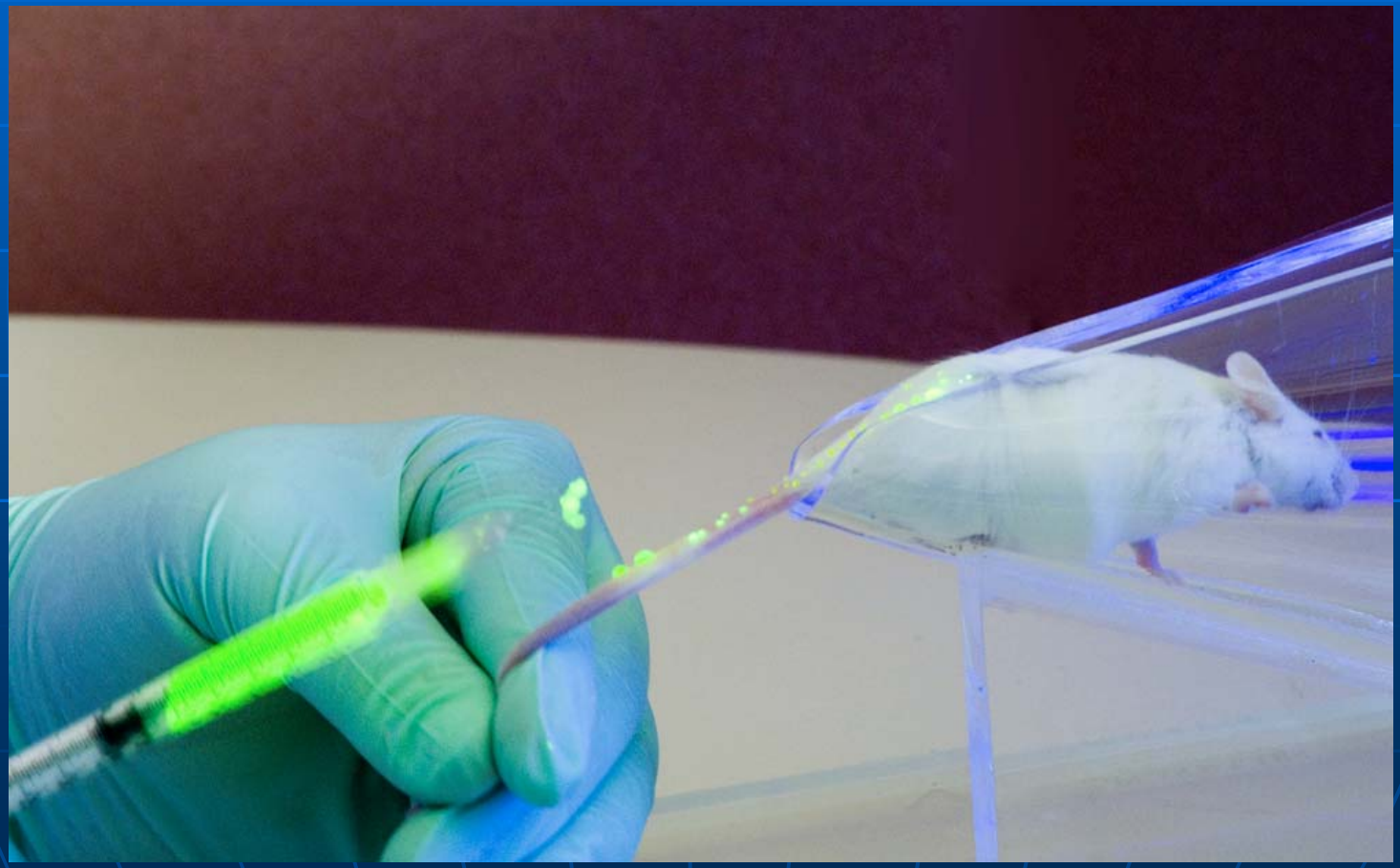
# Restraint Device

- Hands away from action…

# Tail Vein Injection



# Post-Injection Leakage



# Animal Activity and Dispersion of Materials

# Caging

- Automatic watering microisolator

# Acknowledgements


- Bruce Crise
- Joe Kozlovac
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