Consideration of Biosafety Level Assignment for Lentiviral Vectors at Vanderbilt University

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I have not duplicated the discussion of the safety of the various lentiviral vector systems that others have made for this presentation. The Vanderbilt IBC has strongly encouraged all of our researchers to use the third-generation HIV-1 lentiviral vectors, such as the Invitrogen system, already described by Dr. Dewhurst.

This presentation will focus on why we have chosen to not use Biosafety Level 3 containment or even Biosafety Level 2+ (Biosafety Level 2 facilities plus Biosafety Level 3 practices). Rather, we have chosen to stipulate ***enhanced* Biosafety Level 2** containment and have generally assigned ***Animal BSL2*** for any animal work with lentiviral vectors (due to a lack of data on the possibility of shedding from immunodeficient or otherwise modified animals).

A. Assumptions:

1. Assume work with small quantities of wild-type parental strain (if unlikely RCL occurs).
2. Attributes of HIV-1 are listed in attached MSDS. Of note, transmission (Section II) relevant to a research environment is: *"Transmitted from person to person through direct exposure to infected body fluids (blood, semen), unclean needles etc."* Also primary hazards in a laboratory setting (Section VI) are listed as: *"Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosols exposure unknown."* Viability (Section IV) is limited and organism is susceptible to common disinfectants.
3. Assume genetic insert has oncogenic potential if administered to humans.
4. Although unlikely in immunocompetent animals but unknown in immunodeficient or otherwise modified animals, assume animals can shed virus plus insert. Not transmissible between animals. Virus is likely to be viable in bedding no more than 48 hours, probably less. Possible transmission via bites (saliva plus wound); unlikely from scratches (unless subsequently contaminated with viable virus).

B. Assessment:

1. A summary of the differences between BSL2 and BSL3 is provided in Table 1.
2. BSL3 features that would be likely to reduce likelihood of transmission of vector through direct contact with skin or mucous membranes, autoinoculation, or ingestion:
    a. All work conducted in BSCs.
3. **Enhanced** BSL2 features to increase safety of use of lentiviral vectors:
    a. Strict attention to sharps safety. Positive confirmation of the use of safety devices.
    b. Mucous membrane protection (BSC screen; eye, nose, mouth protection; or face shield) for laboratory and animal workers.

4. Additional enhancements:
    a. Notification regarding theoretical mobilization of lentiviral vectors in persons with HIV-1 infection:
        i. Notification in approval letter (goes to all identified research personnel): "*The risk of spreading a vector lentivirus to others following an occupational exposure (e.g., a needlestick or a non-intact skin or mucous membrane exposure) to any lentiviral vector may theoretically be higher for those who are infected with HIV (at the time of a lentivirus vector-inoculating accidental exposure or at a later time). Theoretically, the recombinant lentivirus vector could be mobilized and be transmitted to others in the same manner that HIV-1 is transmitted, if HIV is also present in the body (see Logan, A.C.,* et al., J. Virol. *78: 8421-8436). If a laboratory worker participating in a lentivirus vector research project may have a risk of HIV exposure, it is an option to consider HIV screening at Occupational Health Clinic."*

C. Summary:

1. Even with worst-case assumptions, most Biosafety Level 3 components do not add protection from or prevention of lentiviral exposures. ***Enhanced*** features of BSL2, plus a few additional considerations are warranted and are stipulated for use of lentiviral vectors at Vanderbilt:
    a. All manipulations of lentiviral vector in BSC or with appropriate eye, nose, & mouth or face protection.
    b. Strict attention to sharps safety and positive confirmation of use of safety devices, as applicable.
    c. Notification of theoretical mobilization and offer of HIV screening.
    d. Use of Animal Biosafety Level 2 for animal work.
2. FIV vectors have been classified at BSL2 (with enhanced sharps safety if oncogenic insert) – we have not yet considered the use of FIV in animal models. No other lentiviral vectors have been registered with the Vanderbilt IBC.

Table 1. Summary & comparison of key BSL2 and BSL3 components (derived from NIH Guidelines)

BSL2	BSL3 (Includes parallel BSL2 listing, unless specific BSL3 language provided)
Standard Practices	
Restricted access	
Decontaminate work surfaces	
Decontaminate waste	
No mouth pipetting	
No eating, drinking, etc.	
Handwashing after handling organisms and before exiting laboratory	
Minimize aerosols and splashes	
Experiments of lesser biohazard in carefully demarcated areas	All experiments are conducted at BSL3
	Persons under 16 shall not enter lab
Special Practices	
Specific entry policies	
Hazard warning sign	
Insect & rodent control	
Protective clothing required; removed before exit to non-laboratory areas	
Animals not involved in work not permitted	
Avoid skin contamination; wear gloves	
Sharps safety	
Report spills and exposures to IBC and NIH/OBA	
Medical surveillance, as necessary	Baseline serum collected and stored
Biosafety manual	
	Surgical masks or respirators worn in animal rooms
	Animals housed in partial containment systems (or complete PPE plus shower-out for personnel)
	Vacuum lines protected with HEPA filters and liquid disinfectant traps
Containment Equipment	
BSCs used for aerosol creating procedures or high concentrations/volumes	All work conducted in BSCs. If exhaust discharged into lab, certification annually.
Laboratory Facilities	
Laboratory easily cleaned; impervious benchtops; sturdy furniture	
Handwashing sink	
Fly screens on windows, if appropriate	
Autoclave available	
	Double-door entry
	Water-resistant surfaces; penetrations sealed
	Windows closed and sealed
	Self-closing doors
	Autoclave located preferentially within laboratory
	Non-recirculated, directional airflow into laboratory

Office of Laboratory Security MSDS

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Human Immunodeficiency Virus***

SYNONYM OR CROSS REFERENCE: HIV, AIDS, Acquired Immune Deficiency Syndrome, HTLV III LAV

CHARACTERISTICS: Retroviridae (Lentivirus); ss RNA, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase

SECTION II - HEALTH HAZARD

PATHOGENICITY: Insidious onset with non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue; opportunistic infections and malignant diseases without a known cause for immune deficiency

EPIDEMIOLOGY: First reported in 1981; cases recorded in Americas, Europe, Africa and many other areas; patient categories - homosexually or bisexually active men, drug abusers, Haitian/African emigrants, hemophiliacs, sexual partners of men and women in these categories, infants born to parents in this category

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur

INCUBATION PERIOD: Epidemiologic evidence suggests that duration from exposure to onset of symptoms has a minimum range from 6 months to more than 7 years

COMMUNICABILITY: Period of communicability extends from asymptomatic period through appearance of opportunistic diseases

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Several reverse transscriptase and protease inhibitors now licensed

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

PHYSICAL INACTIVATION: Effectiveness of 56·C - 60·C heat in destroying HIV in serum not certain, however, heating small volumes of serum for 30 min at 56·C before serologic testing reduces residual infectivity to below detectable levels

SURVIVAL OUTSIDE HOST: Drying in environment causes rapid (within several hours) 90-99% reduction in

HIV concentration

SECTION V - MEDICAL

SURVEILLANCE: Serological monitoring for evidence of HIV infection

FIRST AID/TREATMENT: Specific measures for the opportunistic diseases that result from AIDS; "Cocktail" multidrug treatment for HIV

IMMUNIZATION: None available

PROPHYLAXIS: Experimental prophylaxis with AZT/DDI or other appropriate drug

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 5 reported laboratory acquired infections with HIV (splashing of infected materials, inapparent skin exposure, puncture wounds); 18 reported cases in health care workers worldwide

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, CSF, other specimens containing visible blood, unscreened or inadequately treated blood products

PRIMARY HAZARDS: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosols exposure unknown

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and splashing infected materials - virus should be presumed in/on all equipment and devices coming in direct contact with infected materials

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and non-cultured procedures (primary containment devices may be indicated eg. biological safety cabinets) and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV; Biosafety level 3 practices, containment equipment and facilities for all work culturing HIV

PROTECTIVE CLOTHING: Gloves should be worn when handling potentially infectious specimens, cultures or tissues; laboratory coats, gowns or suitable protective clothing should be worn

OTHER PRECAUTIONS: Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal - steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

SECTION IX - MISCELLANEOUS INFORMATION

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LCDC

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