

INTERNATIONAL AIDS VACCINE INITIATIVE

SUPPORT TO RESEARCH AND DEVELOPMENT AT THE INTERNATIONAL AIDS VACCINE INITIATIVE

ENVIRONMENTAL MANAGEMENT PLAN

November 2018

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1. Introduction

1.1 Background

The International AIDS Vaccine Initiative (IAVI) is a nonprofit scientific organization whose mission is to develop vaccines and other biomedical innovations that prevent HIV infection. Since its founding in 1996, IAVI has provided scientific, research and development and policy leadership to address the needs of communities and key populations at risk for HIV infection around the world. IAVI works with more than 100 academic, industry, government, civil society, clinical and community partners in more than 25 countries. IAVI is committed to supporting the broad field of HIV vaccine research and to fostering collaborations that accelerate the development and availability of new prevention tools. In pursuit of our goals, we work to catalyze and support novel partnership models that engage partners from both the public and private sectors across the product development continuum. IAVI's global reach, including its clinical research network in five countries in sub-Saharan Africa and in India, has allowed IAVI to make fundamental contributions to understanding of the epidemiology, transmission, natural history, virology and immunology of HIV infection. This work played a key role in facilitating the structure-based design of promising HIV vaccine candidates as well as the discovery of "broadly neutralizing antibodies" that are now being advanced as promising approaches for HIV prevention. IAVI's integrated capabilities in vaccine discovery, development and clinical research take advantage of biopharmaceutical industry expertise to accelerate the development and testing of novel HIV vaccine candidates. In addition to its core HIV vaccine effort, IAVI is working to amplify its global health impact by working with partners to address other urgent unmet public health needs, such as vaccines for other infectious diseases and where our existing technologies, assets and experience can add unique value.

1.2 Project Description

The work plan summarized below includes *in vitro* laboratory research on: 1) detailed immunologic and transcriptomics analysis on samples collected from studies funded by IAVI and other collaborating partners; 2) applied research aimed at developing a cell line that will support GMP-manufacturing of G-pseudotyped VSVΔG-Env.BG505; and, 3) construction and characterization of two VSV-like vectors.

1. Complete assessment of samples collected from the preclinical immunogenicity and efficacy study (Study #1701) in which the VSVΔG-Env.BG505/1 vaccine was compared against the original VSVΔG-Env.BG505. Conduct standard immunologic and virologic assays, more advanced characterization of anti-Env serum antibody binding specificities and expanded analysis that includes assessment of transcriptomics profiles. Deliverables include:
 - a. Complete sample analysis (immunologic and virologic) associated with standard monitoring
 - i. Sample processing
 - ii. Quantification of Env binding antibodies (direct ELISA)
 - iii. Quantification of Env-specific and VSV N-specific T-cells (flow cytometry)
 - iv. Analysis of simian-human immunodeficiency virus (SHIV) viremia (virus load analysis)
 - b. Complete more advanced characterization of the Env-specific serum antibodies induced by vaccination
 - i. Conduct capture ELISA to determine if serum antibodies bind native-like HIV Env trimer and isolated Env domains
 - ii. Develop a monoclonal antibody binding competition assay and investigate Env epitope specificity
 - iii. Develop a cell-binding assay to quantify serum antibodies that recognize Env on target cells

- iv. Develop capability to amplify and sequence HIV Env genes from animals infected with SHIV and determine if immune pressure, due to vaccination, can be detected as changes in the Env gene
 - c. Analyze blood transcriptomic profiles at select time points after vaccination
 - i. Complete RNA-seq to produce genome-wide gene expression data
 - ii. Conduct computational analysis to determine how vaccination affects modular gene sets such as those connected to B- and T-cell responses, the interferon response, or the inflammatory response
 - d. If VSVΔG-Env.BG505/1 produces preclinical data superior to the original VSVΔG-Env.BG505 chimeric virus vaccine, derive a pre-master virus seed (pre-MVS) that will support future cGMP manufacturing.
 - i. Produce a pre-MVS under well-documented conditions
 - ii. Confirm the characteristics of the pre-MVS and verify the stock is free of microorganism contamination
 - iii. Develop a plan with the WB for advancing the candidate beyond the preclinical stage
2. Advance G-pseudotyping technology to support VSVΔG-Env.BG505 product development. Currently, the G-pseudotyping process relies on transient expression of G in large-scale cell cultures that are then infected with VSVΔG-Env.BG505. The method supports preclinical research, but it is relatively complex, labor-intensive, and not suitable for development of a reliable cGMP manufacturing process.

Development of a ‘pseudotyping’ cell line that expresses VSV G will enable a more practical path forward to a vaccine production process. Because G has cytotoxic properties, very few cell lines have been developed that express the VSV glycoproteins from VSV IND or NJ. Moreover, very little work on pseudotyping cell lines has been done using cell substrates that are acceptable for vaccine manufacturing (i.e. Vero). Thus, to ensure progress on this technically challenging objective, we will investigate four paths for making a pseudotyping cell line.

As part of the earlier stage of the IAVI/WB/Japan ODA program, we have done some initial work on modifying the Vero cell line to express the VSV G glycoprotein along with the human CD4 and CCR5 coreceptors needed to propagate VSVΔG-Env.BG505. First, a DNA expression vector was developed that expressed a ‘tracistronic’ mRNA encoding CD4, CCR5, and VSV G under control of a modified cellular promoter we have used before to develop Vero cell lines. A drug-selection marker under the control of a separate transcription unit also was included in the plasmid DNA. The DNA expression vector was electroporated into Vero cells and multiple stable drug-resistant cell lines were isolated after which they were analyzed for their ability to produce G-pseudotyped VSVΔG-Env.BG505. The results showed that the cell lines did produce G-pseudotyped virus, but the yields were low. Importantly, this initial result provided evidence that a G-pspseudotyping cell line was feasible, although further development and innovation would be necessary.

This project will progress through a number of stages. As noted above, we will initially test four approaches for cell line development using a Vero cell substrate as the starting material. Each approach will be advanced to the stage where technical feasibility is evaluated. In the next stage, cell lines that appear promising will be used to make vaccine to determine if virus yields are adequate and whether the vaccine material has the expected characteristics. Finally, if a stable pseudotyping cell line is developed that enables reproducible preparation of vaccine, we will conduct a preclinical immunogenicity and efficacy study to directly compare vaccine prepared by our research method to

vaccine produced by the pseudotyping cell line. Because development of the pseudotyping cell line is very important for advancing the VSV Δ G-Env.BG505 vaccine product for use in humans, we anticipate that additional preclinical evaluation of vaccine produced with these cells will involve participation of other partners that have a vested interest in the vaccine candidate (BMGF and NIH).

Within the three-year scope of this program we anticipate that the following deliverables will be produced:

- a. Technical feasibility of four pseudotyping cell line approaches will be tested:
 - i. A modified tricistronic CD4-CCR5-G DNA expression vector introduced into Vero cells
 - ii. A VSV G DNA expressing construct introduced into our existing Vero-CD4/CCR5 cell line
 - iii. A DNA expression construct encoding an alternative G glycoprotein that is less cytotoxic (i.e. cocal virus G) introduced into our existing Vero-CD4/CCR5 cell line
 - iv. A DNA expression construct encoding an alternative viral glycoprotein that has been used to make stable cells lines before (i.e. LCMV GP or LASV GP) and supports VSV pseudotyping
 - b. As the lead pseudotyping cell line emerges, vaccine material will be produced and subjected to thorough analytics to show that it has properties comparable to the research vaccine shown to be efficacious in earlier studies.
 - c. Produce a GMP compliant cell line for the selected cell line.
3. Advance development of additional VSV-like vaccine vectors to enable broader application of the chimeric virus vaccine platform to other viral diseases. This work will be a continuation of effort initiated in an earlier phase of the IAVI/WB/Japan/ODA program on the VSV_{NJ} vector. Deliverables will include:
- a. A characterized VSV_{NJ} Δ G-Env.BG505 vector shown to have replicative capacity equivalent to the original VSV Δ G-Env.BG505 counterpart based on VSV_{IND}.
 - b. In addition to VSV_{NJ}, a second new vector will be developed and characterized based on an immunologically distant VSV-like virus to be determined after database sequence comparison. Possibilities for this new vector include VSV-like viruses Cocal virus, Carajas virus, Maraba virus or other more immunologically distant relatives.
 - c. The two new vectors based on VSV_{NJ} and a VSV-like vector will be advanced to the stage where we demonstrate that vaccine material can be produced. This will position us to consider a preclinical studies in which we can address important questions about the effect of anti-VSV_{IND} vector immunity affecting repeated vaccination with VSV_{IND} vector or whether changing the vector genetic background is beneficial. Importantly, this will also produce two new vectors that are suitable for development of chimeric virus vaccines against a larger range of viral pathogens.

Non - Research and Development Component

IAVI/CEPI/GHIT collaborative work in Japan

At the occasion of the first UHC Forum in Tokyo, Japan, in December 2017, IAVI, CEPI and GHIT Fund signed a memorandum of understanding (MOU) with the goal of strengthening coordination among the three organizations in Japan to further promote and enhance the investments in their program. More

specifically, the three organizations committed to develop and implement a set of activities to strengthen their coordination of their programs to promote further engagements from the Japanese scientific community, private sector and other stakeholders.

2. Policy and Legal Framework

2.1 World Bank Safeguard Policies

The World Bank's policy on Environmental Assessment (OP/BP 4.01) is triggered for this project and the project is classified as Category 'B', with preparation of an Environmental Management Plan (EMP) identified as the appropriate safeguard instrument to manage adverse environmental and social risks and impacts.

OP/BP 4.01 on Environmental Assessment is triggered if a project is likely to have significant adverse environmental impacts in its area of influence. Category B projects have limited adverse environmental or social risks and/or impacts that are few in number, generally site-specific, largely reversible, and readily addressed through mitigation measures.

The project will also comply with the World Bank Environmental Health and Safety Guidelines comprising of both the General Guidelines and the relevant Industry Sector Guidelines. Both contain performance levels and specific parameters considered achievable in terms of processes, descriptions, and suggested good practices. The General Guidelines contain requirements and good practice on Environmental, Occupational Health and Safety, Community Health and Safety and other aspects of project implementation. The industry-specific standards cover a number of different sectors including Pharmaceuticals and Biotechnology Manufacturing include information relevant to pharmaceuticals and biotechnology manufacturing facilities. They cover the production of active pharmaceutical ingredients and secondary processing, including intermediates, formulation, blending, and packaging, and related activities research, including biotechnology research and production. To the extent that any of these are relevant, the project will also need to comply with the guidelines.

The EMP, as the identified safeguard instrument, should examine the project's potential negative and positive environmental impacts and recommends any measures needed to prevent, minimize, mitigate, or compensate for adverse impacts.

2.2 Legal Framework

Although the project will not support the procurement of Non-Human Primates (NHPs), animal subjects may be used for the vaccine research to be undertaken as part of the project. In operating research laboratories and conducting research on animal subjects, including NHPs, IAVI complies with all relevant and applicable laws and regulations of the City of New York, the State of New York and the United States, including the U.S. Animal Welfare Act and OSHA regulation 1910.1450 on occupational exposure to hazardous chemicals in laboratories. In addition, the location of the IAVI vivarium (SUNY) maintains animal welfare accreditations from the U.S. Department of Agriculture and AAALAC International, the latter being a voluntary third-party animal welfare accreditation standard for use of animals in scientific research.

3. Environmental Management Plan

3.1 Risk and Impact Identification

Key environmental and social risks and impacts relating to IAVI's activities under this project include: biosafety; occupational health and safety, including exposure to hazardous materials and biohazards; management of hazardous materials; and storage and disposal of hazardous waste and biohazardous/medical waste.

Biosafety risks are particularly high, relating exposure of AIDS culture by researchers, AIDS vaccine prototype by production facility personnel, or HIV infected blood by such personnel at the clinics as nurses, physicians, laboratory analysts, technicians, and other health workers. These exposures may result through an intact or broken skin or a puncture wound, or through the eyes or other mucous membranes such as nose and mouth. Sharps or broken glass contribute to injuries leading to human exposure. Another area of biosafety risk is associated with the handling of animals, including non-human primates (NHPs), during testing and disposal of animal carcasses.

3.2 Management Program

IAVI's existing environmental, health and safety management program includes documented specific management plans and standard operating procedures (SOPs) that address management of key risks and impacts, including a chemical hygiene plan, a biosafety and security plan, a biosafety manual, a safety SOP, a safety audit checklist, a post exposure plan, and biosafety objectives, among others.

The following specific management plans have been developed and implemented at IAVI to manage key risks and impacts, with samples of these plans annexed to the EMP:

- Chemical Hygiene Plan (Annex I)
- Laboratory Biosafety and Security Plan (Annex II)
- Biosafety Manual
- Post Exposure Plan for the Occupational Exposure to Simian Immunodeficiency Virus (SIV) and Simian-Human Immunodeficiency Virus (SHIV) (Annex III)

The following environment, health and safety SOPs have been developed and implemented by IAVI:

0024: Chemical decontamination of biohazardous materials
0026: Safe handling of ultra-low temperature freezers
0027: Working with Human and Animal Cell Cultures
0028: Chemical Spills and Exposure to Hazardous Chemicals
0029: Disposal of Chemical Waste and Empty Chemical Bottles
0030: Storage Use and Disposal of Paraformaldehyde and Formalin
0031: Use of Liquid Waste Vacuum Flask
0032: Use and disposal of ethidium bromide
0033: Use of dry ice
0034: Transport and shipping of Biohazardous Material
0039: Liquid Biohazardous Waste Disposal
0040: Handling of E. Coli Cultures

0041: Exposure to Infectious Agents
0042: Solid Biohazardous Waste Disposal
0043: Standard BSL2 Microbiological Practices
0045: Safe handling broken glass and biohazard sharps
0046: Disinfection of biological spills
0089: Post exposure plan for SIV and SHIV
0110: Preparation of Disinfectant Solutions
0111: Operation of Autoclave
0011: PPE & Signage Requirements

4. Implementation Arrangements and Monitoring

IAVI's management program is managed by a safety committee comprised of nine members with representatives from each of the laboratory research teams that meets quarterly and is responsible for training, recording and monitoring of incidents and revisions to specific management plans and SOPs. Each specific management plan and SOP includes procedures and responsibilities for surveillance, monitoring and reporting on performance related to the relevant risks and impacts for that plan or procedure. In addition, IAVI maintains an audit checklist (Annex 4) to conduct monthly spot checks of work areas and implementation of environment, health and safety measures. The safety committee collects and compiles relevant data for regular review at the quarterly meetings.

The work program and action items for the safety committee are organized and managed through an action plan (Table 1) that is revised and updated regularly

Table 1. Sample Safety Committee Action Plan

Task	2019												2020	
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Send out safety audit schedule (and reminders) for year														
Create safety shower flush schedule for year														
Create biohazard waste schedule for year														
Assign chemical waste pickup point person for year														
Schedule tentative quarterly safety meetings														
Remind DDL'ers and managers of annual safety goal req.														
Track staff safety compliance (SOPs, training, audit sign-offs, etc.														
First aid, biological spill, chem spill, NHP kit check (Contents/ Exp. Dates)														
Schedule fire extinguisher annual inspection														
OSHA 300 Logs (one for BAT and one for Incubator)	For 2019												For 2020	
OSHA 300A Summary Forms (one for BAT and one for Incubator)	Post 2018												Post 2019	
Review/revise annual safety training slides														
Annual Safety Training														
Safety SOP revisions* (every other year)														
Safety SOP training** (every other year)														
Chemical Inventory (every other year)														
CPR-AED re-training (every other year)														
Dangerous goods shipping training (every other year)														

ANNEXES

ANNEX 1

Sample Chemical Hygiene Plan

1. Background Information

On 31 January, 1990 the Occupational Safety and Health Administration (OSHA) promulgated a final rule for occupational exposure to hazardous chemicals in laboratories. The standard was incorporated into Occupational Exposure to Hazardous Chemical for *IAVI Vaccine Design and Development Laboratory* in July of 2009. Compliance will be enforced by IAVI Chemical Hygiene Officer and his/her designees as well as Quality Assurance. Included in the standard, is a requirement for all employers covered by the standard to develop and carry out the provisions of a Chemical Hygiene Plan (CHP).

A CHP is defined as a written program which sets forth procedures, equipment, personal protective equipment and work practices that are capable of protecting employees from the health hazards presented by hazardous chemicals used in that particular workplace. Components of the CHP must include standard operating procedures for safety and health, criteria for the implementation of control measures, measures to ensure proper operation of engineering controls, provisions for training and information dissemination, permitting requirements, provisions for medical consultation, designation of responsible personnel, and identification of particularly hazardous substances.

This plan is the Chemical Hygiene Plan developed for *IAVI Vaccine Design and Development Laboratory*. All laboratory personnel must know and follow the procedures outlined in this plan. All operations performed in the laboratory must be planned and executed in accordance with the enclosed procedures. In addition, each employee is expected to develop safe personal chemical hygiene habits aimed at the reduction of chemical exposures to themselves and coworkers.

This document was developed to comply with paragraph (e) of the OSHA 1910.1450 regulation. *IAVI Vaccine Design and Development Laboratory* will maintain facilities and procedures in laboratories compatible with current knowledge and regulations in laboratory safety. This CHP will be reviewed, evaluated and updated at least annually and is readily available to employees, their representatives and any representative for OSHA.

2. Definitions

Departmental Organization

Currently, there are three active R&D programs at the IAVI Vaccine Design and Development Laboratory: Candidate Design, Candidate Evaluation and Candidate Development. Each program is headed by a Program Director.

Chemical Hygiene Officer (CHO)

A Chemical Hygiene Officer is appointed by the Chair of Safety Committee of the Laboratory. The Chemical Hygiene Officer is responsible for the maintenance of the Chemical Hygiene Plan and Material Safety Data Sheet (MSDS) database and file.

Safety Person

Chair of the Safety Committee shall appoint a safety person responsible for chemical safety in each respective program.

Organization of Chemicals

The National Fire Rating System has been adopted as the standard for the *IAVI Vaccine Design and Development Laboratory*. The National Fire Rating System uses a color-coded alpha numeric description to identify hazards associated with health, flammability, reactivity and special notice hazards. The hazards for about 1,300 chemicals are described in the National Fire Rating System Reference Guide. For a description of safe chemical storage contact the Chemical Hygiene Officer.

Material Safety Data Sheet (MSDS) Database and File

MSDSs provide basic information about the safety and health hazards posed by a chemical and precautions to take when using it (see Appendix A). Hard copies of MSDSs of all chemicals stored or used must be kept on file readily accessible to all laboratory personnel. A departmental MSDS file (hard copy) is located in hallway 854. A database listing the MSDSs organized by location, manufacturer and product name is available on the Design Lab Portal on SharePoint in PDF format. The Principal Investigator may elect to maintain a separate file of MSDSs for their chemicals in their laboratories.

3. Standard Operating Procedures for Laboratory Chemicals

Chemical Procurement

The decision to procure a chemical shall be a commitment to handle and use the chemical properly from initial receipt to ultimate disposal.

Information on proper handling, storage and disposal shall be known to all involved personnel prior to the procurement of the chemical. Chemicals utilized in the laboratory shall be those which are appropriate for the ventilation system.

All chemicals shall be received centrally in *IAVI Vaccine Design and Development Laboratory*. Personnel who receive chemical shipments shall be knowledgeable of the proper procedures for receipt. Chemical containers shall not be accepted without proper labels, material safety data sheets and packaging in accordance with all appropriate regulations. All chemical shipments shall be dated when received and opened. 8

Chemicals shall be entered into the laboratory inventory system by the laboratory Safety Person and the MSDSs shall be entered into the MSDS database by the Safety Person or the CHO.

Chemical Storage

Guidelines for proper segregation of incompatible chemicals can be found in Appendix B.

Received chemicals shall be immediately moved to the designated storage area. Large glass containers shall be placed in carrying containers or shipping containers during transportation.

The storage area shall be well-illuminated, with all storage maintained below eye level. Large bottles (greater than 1 liter) shall be stored no more than two feet from ground level.

Chemicals shall be segregated by hazard classification and compatibility in a well-identified and ventilated area.

Mineral acids should be separated from flammable and combustible materials. Separation is defined by NFPA 49 as storage within the same fire area but separated by as much space as practicable or by intervening storage from incompatible materials.

Acid-resistant trays should be placed under bottles of mineral acids.

Acid-sensitive materials such as cyanides and sulfides shall be separated from acids or protected from contact with acids.

Highly toxic chemicals or other chemicals whose containers have been opened shall be stored in unbreakable secondary containers.

When chemicals are taken from the storage area, they shall be placed in an outside container or bucket.

Storage of chemicals at the lab bench or other work areas shall be limited to those amounts necessary for one operation or one day's worth of work. The container size and the amounts of chemicals at the lab bench shall be as small as practical.

Stored chemicals shall be examined at least annually by the safety person for replacement, deterioration, and container integrity. The inspection should determine whether any corrosion, deterioration, or damage has occurred to the storage facility as a result of leaking chemicals.

Periodic inventories of chemicals outside the storage area shall be conducted by the safety person. Unneeded items shall be properly discarded.

Chemical Handling

Each laboratory employee with the training, education and resources provided by his or her supervisor, shall develop and implement work habits consistent with this Chemical Hygiene Plan to minimize personal and coworker exposure to the chemicals in the laboratory.

Based on the realization that all chemicals inherently present hazards in certain conditions, exposure to all chemicals shall be minimized. General precautions which shall be followed for the handling and use of all chemicals are:

- The intent and procedures of this Chemical Hygiene Plan shall be continuously applied.
- Skin contact with all chemicals shall be avoided.
- All employees shall wash all areas of exposed skin prior to leaving the laboratory.
- Mouth suction for pipetting or starting a siphon is prohibited.
- Eating, drinking, smoking, gum chewing, or application of cosmetics in areas where laboratory chemicals are present shall be avoided.
- Storage, handling and consumption of food or beverages shall not occur.
- Risk determinations shall be conservative in nature.
- Any chemical mixture shall be assumed to be as toxic as its most toxic component.
- Substances of unknown toxicity shall be assumed to be toxic.
- Laboratory employees shall be familiar with the symptoms of exposure for the chemicals with which they work and the precautions necessary to prevent exposure.

In all cases of chemical exposure, neither the Permissible Exposure Limits (PELs) of OSHA or the Threshold Limit Values (TLVs) of the American Conference of Governmental Industrial

Hygienists (ACGIH) shall be exceeded. The PELs of OSHA are based on the TLVs developed by the ACGIH. A listing of the TLVs can be found in Appendix B of the book *Prudent Practices for Handling Hazardous Chemicals in Laboratories* published by the National Academy Press.

- The engineering controls and safety equipment in the laboratory shall be utilized as described in Sections 4 and 5.
- Specific precautions based on the toxicological characteristics of individual chemicals shall be implemented as deemed necessary.

Laboratory Equipment and Glassware

- Each employee shall keep the work area clean and uncluttered. All chemicals and equipment shall be properly labeled as described in Section 3 (Labeling). At the completion of each work day or operation, the work area shall be thoroughly cleaned and all equipment properly cleaned and stored.

In addition, the following procedures shall apply to the use of laboratory equipment:

- All laboratory equipment shall be used only for its intended purpose, and repaired and replaced as needed.
- All glassware will be handled and stored with care to minimize breakage; all broken glassware will be immediately disposed of in the broken glass container.
- All evacuated glass apparatus shall be shielded to contain chemicals and glass fragments should implosion occur.
- Labels shall be attached to all chemical containers, identifying the contents and related hazards.
- All waste receptacles shall be identified according to the type of waste - biohazard, ordinary waste, waste glass, etc.

Personal Protective Equipment

Personal protective equipment includes appropriate lab coats or gowns, shoes, safety glasses, gloves, etc. Safety glasses meeting ANSI (American National Standards Institute, www.ansi.org) Z87.1 should be used by employees and visitors to the laboratory and will be worn as needed in the laboratory. Contact lenses are prohibited in the laboratory.

- Chemical goggles and/or a full face shield shall be worn during chemical transfer and handling operations as procedures dictate.
- Sandals, perforated shoes and bare feet are prohibited. Safety shoes, per ANSI 47 are required where employees routinely lift heavy objects.
- Lab coats should be worn in the laboratory. Laboratory coats should be laundered on a periodic basis, not to exceed monthly. Laboratory coats shall be removed immediately upon discovery of significant contamination and laundered or disposed of as needed. Lab coats should not be worn outside of the laboratory.
- Appropriate chemical-resistant gloves shall be worn at all times when there may be skin contact with chemicals. Used gloves shall be inspected and washed prior to reuse. Damaged or deteriorated gloves will be immediately replaced. Gloves shall be washed prior to removal from the hands. Disposable gloves shall not be reused.

- Thermal-resistant gloves shall be worn for operations involving the handling of heated materials and exothermic reaction vessels. Thermal-resistant gloves shall be non-asbestos and shall be replaced when damaged or deteriorated.
- Respirator usage shall comply with the OSHA Respiratory Protection Standard, 29 CFR 1910.134. The use of respirators must be determined on an individual basis. The situation must be analyzed, and the risk determined by qualified individuals.

Personal Work Practices

Chemical Hygiene Officer must ensure that each employee knows and follows the rules and procedures established in this plan.

All employees shall remain vigilant to unsafe practices and conditions in the laboratory and shall immediately report such practices and/or conditions to the Principal Investigator. The Principal Investigator must correct unsafe practices and or conditions promptly.

- Long hair and loose-fitting clothing shall be confined close to the body to avoid being caught in moving machine/equipment parts.
- Use only those chemicals appropriate for the ventilation system.
- Avoid unnecessary exposure to all chemicals by any route.
- Do not smell or taste any chemicals.
- Encourage safe work practices in coworkers by setting the proper example. Horseplay is strictly forbidden.
- Seek information and advice from knowledgeable persons, standards and codes about the hazards present in the laboratory. Plan operations, equipment and protective measures accordingly.
- Use engineering controls in accordance with Section 5 of this document.
- Inspect personal protective equipment prior to use and wear appropriate protective equipment as procedures dictate and when necessary to avoid exposure.

Labeling

All containers in the laboratory shall be labeled. This includes chemical containers and waste containers. The label shall be informative and durable, and at a minimum, will identify contents, source, and date of acquisition and indication of hazard.

The labeling program shall be periodically inspected by the Safety Person to ensure that labels have not been defaced or removed.

4. Criteria For Implementation Of Control Measures

Air Sampling

Air sampling for evaluating employee exposure to chemical substances shall be conducted periodically or as specified by specific codes, regulations or IAVI senior management. The sampling shall be done by a qualified air sampling firm.

Housekeeping

Each laboratory worker is directly responsible for the cleanliness of his or her work space, and jointly responsible for common areas of the laboratory. Laboratory management shall insist on the maintenance of housekeeping standards.

- The following procedures apply to the housekeeping standards of the laboratory:
- All spills on lab benches or floors shall be immediately cleaned and disposed of properly. Large spills will necessitate the implementation of the Emergency Action Plan per OSHA 29 CFR 1910.38 and 1910.120. The Chemical Hygiene Officer shall be notified immediately. The Fire Department or local Police should be notified as needed.
- The lab benches shall be kept clear of equipment and chemicals except those necessary for the work currently being performed.
- The work area shall be cleaned at the end of each operation and each shift.
- All apparatus shall be thoroughly cleaned and returned to storage upon completion of usage.
- All floors, aisles, exits, fire extinguishing equipment, eyewashes, and showers, electrical disconnects and other emergency equipment shall remain unobstructed.
- All labels shall face front.
- Chemical containers shall be clean, properly labeled and returned to storage upon completion of usage.
- All chemical wastes will be disposed of in accordance with the Hazardous Materials Policies of IAVI *Vaccine Design and Development Laboratory* (see Appendix C).

Safety and Emergency Equipment

Telephone numbers of emergency personnel, supervisors and other workers as deemed appropriate shall be posted on laboratory doors and Safety Bulletin Board (located in corridor 861). All laboratory personnel will be trained in the proper use of fire extinguishers when hired and annually thereafter. Prior to the procurement of new chemicals, the safety person shall verify that existing extinguishers and other emergency equipment are appropriate for such chemicals.

All employees who might be exposed to chemical splashes shall be instructed in the location and proper usage of emergency showers and eyewashes. The eyewash shall be inspected weekly and emergency shower shall be inspected at least monthly. These inspections shall be performed by the laboratory employees. These inspections shall be in accordance with ANSI Z358.1 and manufacturer's specifications. Records shall be maintained on equipment.

Location signs for safety and emergency equipment have been posted.

5. Engineering Controls

Intent

Engineering controls include electrical switches, thermostats, safety interlocks and other control devices. The engineering controls installed in the laboratory are intended to minimize employee exposure to chemical and physical hazards in the workplace. These controls must be maintained in proper working order for this goal to be realized.

Modification

No modification of engineering controls will occur unless testing indicates that worker protection will continue to be adequate.

Improper Function

Improper function of engineering controls must be reported to the Safety Person immediately. The system shall be taken out of service until proper repairs have been executed.

Usage

All employees shall follow proper work practices when using the engineering controls.

Local Exhaust Ventilation

The following procedures shall apply to the use of local exhaust ventilation, if laboratory ventilation exists:

- Hood fans shall operate when hoods are being used.
- After using hoods, operate the fan for an additional period of time sufficient to clear residual contaminants from the ductwork.
- The ventilation system shall be inspected semi-annually. The duct velocity shall be maintained at 3500 feet per minute, minimum. A record of each inspection shall be maintained by the "responsible person".
- Prior to a change in chemicals or procedures, the adequacy of the ventilation system shall be determined by the "responsible person".

Laboratory Fume Hoods

The laboratory hoods shall be utilized for all chemical procedures which might result in release of hazardous chemical vapors or dust. As a general rule, the hood shall be used for all chemical procedures involving substances which are appreciably volatile and have a permissible exposure limit (PEL) less than 50 ppm. The following work practices shall apply to the use of hoods:

- Confirm adequate hood ventilation performance prior to opening chemical containers inside the hood. An inward flow of air can be confirmed by holding a piece of paper at the face of the hood and observing the movement of the paper.
- Keep the sash of the hood closed at all times except when work is being done inside the hood. When working inside the hood, maintain the sash height as low as possible.
- Storage of chemicals and equipment inside the hood shall be kept to a minimum.
- Minimize interference with the inward flow of air into the hood.
- Leave the hood operating when it is not in active use if hazardous chemicals are contained inside the hood or if it is uncertain whether adequate general laboratory ventilation will be maintained when the hood is non-operational.
- The ventilation system shall be inspected every semi-annually. The hood face velocity shall be maintained between 75 and 125 feet per minute. A record of each inspection shall be maintained by the Safety Person and attach an inspection label. The hood shall not be used as a means of disposal for volatile chemicals.
- Prior to the introduction of new chemicals, the adequacy of hood ventilation systems shall be determined by the Safety Person.

Storage Cabinets

Storage cabinets for flammable and hazardous chemicals will be ventilated as needed.

6. Employee Information And Training

Hazard Information

All employees will be apprised of the hazards presented by the chemicals in use in the laboratory. Each employee shall receive training at the time of initial assignment to the laboratory, prior to assignments involving new exposure situations, and at a regular frequency as determined by the CHO. 11

Safety and Emergency Equipment

Telephone numbers of emergency personnel, supervisors and other workers as deemed appropriate shall be posted on laboratory doors and Safety Bulletin Board (located in corridor 861). All laboratory personnel will be trained in the proper use of fire extinguishers when hired and annually thereafter. Prior to the procurement of new chemicals, the safety person shall verify that existing extinguishers and other emergency equipment are appropriate for such chemicals.

All employees who might be exposed to chemical splashes shall be instructed in the location and proper usage of emergency showers and eyewashes. The eyewash shall be inspected weekly and emergency shower shall be inspected at least monthly. These inspections shall be performed by the laboratory employees. These inspections shall be in accordance with ANSI Z358.1 and manufacturer's specifications. Records shall be maintained on equipment.

Location signs for safety and emergency equipment have been posted.

7. Engineering Controls

Intent

Engineering controls include electrical switches, thermostats, safety interlocks and other control devices. The engineering controls installed in the laboratory are intended to minimize employee exposure to chemical and physical hazards in the workplace. These controls must be maintained in proper working order for this goal to be realized.

Modification

No modification of engineering controls will occur unless testing indicates that worker protection will continue to be adequate.

Improper Function

Improper function of engineering controls must be reported to the Safety Person immediately. The system shall be taken out of service until proper repairs have been executed.

Usage

All employees shall follow proper work practices when using the engineering controls.

Local Exhaust Ventilation

The following procedures shall apply to the use of local exhaust ventilation, if laboratory ventilation exists:

- Hood fans shall operate when hoods are being used.
- After using hoods, operate the fan for an additional period of time sufficient to clear residual contaminants from the ductwork.
- The ventilation system shall be inspected semi-annually. The duct velocity shall be maintained at 3500 feet per minute, minimum. A record of each inspection shall be maintained by the "responsible person".
- Prior to a change in chemicals or procedures, the adequacy of the ventilation system shall be determined by the "responsible person".

Laboratory Fume Hoods

The laboratory hoods shall be utilized for all chemical procedures which might result in release of hazardous chemical vapors or dust. As a general rule, the hood shall be used for all chemical procedures involving substances which are appreciably volatile and have a permissible exposure limit (PEL) less than 50 ppm. The following work practices shall apply to the use of hoods:

- Confirm adequate hood ventilation performance prior to opening chemical containers inside the hood. An inward flow of air can be confirmed by holding a piece of paper at the face of the hood and observing the movement of the paper.
- Keep the sash of the hood closed at all times except when work is being done inside the hood. When working inside the hood, maintain the sash height as low as possible.
- Storage of chemicals and equipment inside the hood shall be kept to a minimum.
- Minimize interference with the inward flow of air into the hood.
- Leave the hood operating when it is not in active use if hazardous chemicals are contained inside the hood or if it is uncertain whether adequate general laboratory ventilation will be maintained when the hood is non-operational.
- The ventilation system shall be inspected every semi-annually. The hood face velocity shall be maintained between 75 and 125 feet per minute. A record of each inspection shall be maintained by the Safety Person and attach an inspection label. The hood shall not be used as a means of disposal for volatile chemicals.
- Prior to the introduction of new chemicals, the adequacy of hood ventilation systems shall be determined by the Safety Person.

Storage Cabinets

Storage cabinets for flammable and hazardous chemicals will be ventilated as needed.

8. Employee Information And Training

Hazard Information

All employees will be apprised of the hazards presented by the chemicals in use in the laboratory. Each employee shall receive training at the time of initial assignment to the laboratory, prior to assignments involving new exposure situations, and at a regular frequency as determined by the CHO. 11

Training

This training shall include methods of detecting the presence of a hazardous chemical, physical and health hazards of chemicals in the lab, and measures employees can take to protect themselves from these hazards. The training shall present the details of the Chemical Hygiene Plan and shall include:

- The contents, location and availability of the Chemical Hygiene Plan.
- Signs and symptoms associated with exposure to the chemicals present in the laboratory (see Appendix D).
- Location and availability of reference material on chemical hygiene (Safety Bulletin Board).
- Training shall be conducted by the Chemical Hygiene Officer or the Safety Person as described in this section.

9. Prior Approval Of Laboratory Activities

Permit System

A permit system is not a reasonable precaution to improve safety in the IAVI Design and Vaccine Development Laboratory. It is more prudent to use adequate training to prevent unforeseen situations.

Off-Hours Work Procedures

Laboratory personnel are not permitted to work after hours in the lab, except when specifically permitted by Laboratory Head.

Sole Occupancy

At no time should work be performed in the laboratory when the only person in the building is the laboratory person performing the work.

Hazardous Work

All hazardous operations are to be performed during a time when at least two personnel are present at the laboratory. At no time shall a laboratory person, while working alone in the laboratory, perform work which is considered hazardous. The determination of hazardous operations shall be made by the Laboratory Head.

Unattended Operations

When laboratory operations are performed which will be unattended by laboratory personnel (continuous operations, overnight reactions, etc.), the following procedures will be employed:

- The Principal Investigator will review work procedures to ensure for the safe completion of the operation.
- An appropriate sign will be posted at all entrances to the laboratory.
- The overhead lights in the laboratory will be left on.
- Precautions shall be made for the interruption of utility service during the unattended operation (loss of water pressure, electricity, etc.).
- The person responsible for the operation will return to the laboratory at the conclusion of the operation to assist in the dismantling of the apparatus.

10. Medical Consultations And Examinations

An opportunity to receive medical attention is available to all employees who work with hazardous chemicals in the laboratory. The opportunity for medical attention will be made available to employees under the following circumstances:

- Whenever an employee develops signs or symptoms associated with a hazardous chemical to which the employee may have been exposed in the laboratory,
- Whenever an event takes place in the laboratory such as a spill, leak, explosion or other occurrence resulting in the likelihood of a hazardous exposure the employee will be provided an opportunity for medical consultation for the purpose of determining the need for medical examination.
- These medical consultations and examinations shall be provided without cost to the employees, without loss of pay and at a reasonable time and place.
- These medical consultations and examinations shall be administered by or under the direct supervision of a licensed physician

11. Chemical Hygiene Responsibilities

Laboratory Head

The Laboratory Head has the ultimate responsibility for chemical hygiene throughout the laboratories and will provide continued support for chemical hygiene and appoints the Chemical Hygiene Officer.

Laboratory Directors

Each Laboratory Director has the responsibility for chemical hygiene within their department and will provide support for chemical hygiene.

Principal Investigators

The Principal Investigators are responsible for chemical hygiene within their laboratory and shall appoint a Safety Person.

Chemical Hygiene Officer

The Chemical Hygiene Officer shall:

- Work with the Biosafety Officer and scientific staff to develop and implement appropriate chemical hygiene policies and practices.
- Monitor the use of chemicals in the lab, including determining that facilities and training levels are adequate for the chemicals in use.
- Help Principal Investigators develop precautions and adequate facilities.
- Review and improve the Chemical Hygiene Plan on an annual basis.
- Maintain overall responsibility for the safe operation of the laboratories. Ensure that workers know and follow the chemical hygiene rules,
- Ensure that appropriate training has been provided to employees,
- Monitor the waste disposal program,
- Maintain the MSDS database and file.

Safety Person

The responsibilities of the Safety Person overlap and integrate with the responsibilities of the Chemical Hygiene Officer and shall include:

- Monitor the use of chemicals in the lab, including determining that facilities and training levels are adequate for the chemicals in use.
- Ensure that workers know and follow the chemical hygiene rules,

- Ensure that appropriate training has been provided to employees,
- Monitor the waste disposal program,
- Maintain the chemical inventory.

Laboratory Workers

The laboratory workers are individually responsible for:

- Planning and conducting each laboratory operation in accordance with the Chemical Hygiene Plan,
- Developing good personal chemical hygiene habits.

12. Special Precautions

When laboratory procedures change to require the use of additional classifications of chemicals (embryotoxins, teratogens, carcinogens, etc.), additional special precautions shall be implemented as deemed necessary by the Chemical Hygiene Officer. A list of these chemicals is included in Appendix E.

Working with Embryotoxins (Reproductive Toxins) and Carcinogens

Women of child-bearing age will handle embryotoxins only in a hood with confirmed satisfactory performance and will use protective equipment to prevent skin contact as prescribed by the Principal Investigator and Chemical Hygiene Officer.

Embryotoxins will be stored in adequately ventilated areas in unbreakable secondary containers.

The Principal Investigator and Chemical Hygiene Officer will be notified of spills and other exposure incidents. A physician will be consulted when appropriate.

Working with Chemicals of Moderate Chronic or High Acute Toxicity

Areas where these chemicals are stored and used are of restricted access and have special warning signs. Gloves and long sleeves will be used. Hands and arms will be washed immediately after working with these chemicals.

Two people will always be present during work with these chemicals.

13. Recordkeeping

Accident investigations will be conducted by the Biosafety Officer in cooperation with the Laboratory Head with assistance from other personnel as deemed necessary.

Accident reports will be rewritten and retained in the individual's personnel file. Medical records for employees exposed to hazardous chemicals and/or harmful physical agents will be maintained for the duration of employment and kept in individual's personnel file, per 29 CFR 1910.20. MSDS(s) of chemicals involved in the accident will be included in the report.

Records of inspections of equipment will be maintained for five years.

Records of employee training will be maintained for five years.

14. Chemical Spills, Releases And Accidents

In the event of a chemical spill, release or other accident, immediately notify the Safety Person, Chemical Hygiene Officer. If they are not available directly call 911.

15. Annual Chemical Hygiene Plan Audit

The Chemical Hygiene Officer will conduct an audit of all phases of the Chemical Hygiene Plan each year. Results will be provided to the Biosafety Officer and Laboratory Head. Laboratory directors are responsible for taking corrective action.

16. References And Recommended Reading

National Research Council, Prudent Practices for Handling Hazardous Chemicals in Laboratories, National Academy Press, Washington, D.C.1981.

National Research Council, Prudent Practices for Disposal of Chemicals from Laboratories, National Academy Press, Washington, D.C., 1983.

Freeman, N.T., Introduction to Safety in the Chemical Laboratory, National Academy Press, 1982.

Manufacturing Chemists' Association, Inc., Guide for Safety In The Chemical Laboratory, D. Van Nostrand Company, Inc., 1954.

Green, Michael E., Safety in Working with Chemicals, MacMillan Publishing Co., Inc., 1978. 13

Pipitone, Davin A. Safe Storage of Laboratory Chemicals, Wiley & Sons, Inc., 1984

Code of Federal Regulations, 29 CFR part 1910 subpart Z section 1910.1450, Occupational Exposure to Hazardous Chemicals in Laboratories, 1990.

Pulvere-Edwards, Theresa A. and Peter L. Pingerelli. 1994. A Guide to the Handling of Hazardous Compounds. Calbiochem-Novabiochem International.

ANNEX 2

Sample Laboratory Biosafety and Security Plan

Overview

The information contained in this manual is the basis for IAVI's Biosafety portion of the Biosafety and Security Plan and based on recommendations from the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition.

http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf

This manual contains sufficient information for employees to deal with biosafety and containment issues in the AIDS Vaccine Design & Development Laboratory (DDL). This manual, as well as the Biosafety and Security Plan, will be reviewed biennially and revised as necessary. Comprehensive safety training for all DDL employees will be required and conducted annually to ensure the effectiveness of the plan.

Although this manual describes Biosafety Levels 1, 2 and 3, the general laboratory area is being designated as BSL-2 and those procedures and guidelines will be highlighted in this manual.

All aspects of this manual and the Biosafety and Security Plan will be enforced by the laboratory management and Safety Committee.

PART I - BIOSAFETY GUIDELINES

General principles

Classification of infective microorganisms by risk group

Risk Group 1 (no or low individual and community risk)

A well-characterized agent not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel or the environment.

Risk Group 2 (moderate individual risk, low community risk)

A pathogen that represents moderate potential hazard to personnel and environment but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited.

Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease. Effective treatment and preventive measures are available.

Risk Group 4 (high individual and community risk)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Laboratory facilities are designated as basic – Biosafety Level 1, basic – Biosafety Level 2, containment – Biosafety Level 3, and maximum containment – Biosafety Level 4. Biosafety level designations are based on a composite of the design features, construction, containment facilities, equipment, practices and operational procedures required for working with agents from the various risk groups.

Factors that determine the risk group:

1. Pathogenicity of the organism.
2. Mode of transmission and host range of the organism. These may be influenced by existing levels of immunity in the local population; density and movement of the host population, presence of appropriate vectors, and standards of environmental hygiene.
3. Local availability of effective preventive measures. These may include: prophylaxis by immunization or administration of antisera (passive immunization); sanitary measures, e.g. food and water hygiene; control of animal reservoirs or arthropod vectors.
4. Local availability of effective treatment. This includes passive immunization, post exposure vaccination and use of antimicrobials, antivirals and chemotherapeutic agents, and should take into consideration the possibility of the emergence of drug-resistant strains.

The assignment of an agent to a biosafety level for laboratory work must be based on a risk assessment. Such an assessment will take the risk group as well as other factors into consideration in establishing the appropriate biosafety level. For example, an agent that is assigned to Risk Group 2 may generally require Biosafety Level 2 facilities, equipment, practices and procedures for safe conduct of work. There are certain examples when the same agent may be assigned to Biosafety level 3 (e.g. genetic manipulation, large volumes of fluids, high amounts of aerosols, etc.).

Microbiological risk assessment

The foundation of the practice of biosafety is risk assessment. While there are many ways to evaluate and describe risk assessment, the most important component is professional judgment. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, and the containment equipment and facilities available. The laboratory director or principal investigator is responsible for ensuring that adequate and timely risk assessments are performed, and for working closely with the institution's safety committee and biosafety personnel to ensure that appropriate equipment and facilities are available to support the work being considered. Once performed, risk assessments should be reviewed routinely and revised when necessary, taking into consideration the acquisition of new data having a bearing on the degree of risk and other relevant new information from the scientific literature.

One of the most helpful tools available for performing a microbiological risk assessment is the listing of risk groups for microbiological agents.

Other factors that should be considered, as appropriate, include:

1. Pathogenicity of the agent and infectious dose
2. Potential outcome of exposure
3. Natural route of infection
4. Other routes of infection, resulting from laboratory manipulations (parenteral, airborne, ingestion)

5. Stability of the agent in the environment
6. Concentration of the agent and volume of concentrated material to be manipulated
7. Presence of a suitable host
8. Information available from animal studies and reports of laboratory-acquired infections or clinical reports
9. Laboratory activity planned (sanitation, aerosolization, centrifugation, etc.)
10. Any genetic manipulation of the organism that may extend the host range of the agent or alter the agent's sensitivity to known, effective treatment regimens
11. Local availability of effective prophylaxis or therapeutic interventions.

On the basis of the information ascertained during the risk assessment, a biosafety level can be assigned to the planned work, appropriate personal protective equipment selected, and standard operating procedures (SOPs) incorporating other safety interventions developed to ensure the safest possible conduct of the work.

The risk assessment procedure described above works well when there is adequate information available. However, there are situations when the information is insufficient to perform an appropriate risk assessment, for example, with clinical specimens or epidemiological samples collected in the field. In these cases, it is prudent to take a cautious approach to specimen manipulation.

Standard precautions should always be followed, and barrier protections applied (gloves, gowns, eye protection), whenever specimens are obtained. Biosafety Level 2 practices and procedures should be the minimum requirement for handling specimens. Transport of specimens should follow national and/or international rules and regulations.

Some information may be available to assist in determining the risk of handling these specimens:

1. Medical data on the specimen source
2. Epidemiological data (morbidity and mortality data, suspected route of transmission, other outbreak investigation data)
3. Resources and links for locating risk groups:

ABSA Risk Group Database

<http://www.absa.org/riskgroups/index.html>

NIH Guidelines, Appendix B (Classification of Human Etiologic Agents on the Basis of Hazard)

http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html#_Toc351276291

Genetically Modified Agent Hazards

The identification and assessment of hazardous characteristics of genetically modified agents involve consideration of the same factors used in risk assessment of the wild-type organism. It is particularly important to address the possibility that the genetic modification could increase an agent's pathogenicity or affect its susceptibility to antibiotics or other effective treatments. The risk assessment can be difficult or incomplete, because important information may not be available for a newly engineered agent. Several

investigators have reported that they observed unanticipated enhanced virulence in recent studies with engineered agents. These observations give reason to remain alert to the possibility that experimental alteration of virulence genes may lead to increased risk. It also suggests that risk assessment is a continuing process that requires updating as research progresses.

The NIH Guidelines are the key reference in assessing risk and establishing an appropriate biosafety level for work involving recombinant DNA molecules. The purpose of the NIH Guidelines is to promote the safe conduct of research involving recombinant DNA. The guidelines specify appropriate practices and procedures for research involving constructing and handling both recombinant DNA molecules and organisms and viruses that contain recombinant DNA. They define recombinant DNA as a molecule constructed outside of a living cell with the capability to replicate in a living cell. The NIH Guidelines explicitly address experiments that involve introduction of recombinant DNA into Risk Groups 2, 3, and 4 agents, and experiments in which the DNA from Risk Groups 2, 3, and 4 agents is cloned into nonpathogenic prokaryotic or lower eukaryotic host-vector systems. Compliance with the NIH Guidelines is mandatory for investigators conducting recombinant DNA research funded by the NIH or performed at, or sponsored by, any public or private entity that receives any NIH funding for recombinant DNA research. Many other institutions have adopted these guidelines as the best current practice.

The NIH Guidelines were first published in 1976 and are revised on an ongoing basis in response to scientific and policy developments. They outline the roles and responsibilities of various entities affiliated with recombinant DNA research, including institutions, investigators, and the NIH. Recombinant DNA research subject to the NIH Guidelines may require: 1) approval by the NIH Director, review by the NIH Recombinant DNA Advisory Committee (RAC), and approval by the IBC; or 2) review by the NIH Office of Biotechnology Activities (OBA) and approval by the IBC; or 3) review by the RAC and approvals by the IBC and Institutional Review Board; or 4) approval by the IBC prior to initiation of the research; or 5) notification of the IBC simultaneous with initiation of the work. It is important to note that review by an IBC is required for all non-exempt experiments as defined by the NIH Guidelines.

The NIH Guidelines were the first documents to formulate the concept of an IBC as the responsible entity for biosafety issues stemming from recombinant DNA research. The NIH Guidelines outlines the membership, procedures, and functions of an IBC. The institution is ultimately responsible for the effectiveness of the IBC and may define additional roles and responsibilities for the IBC apart from those specified in the NIH Guidelines. See Appendix J for more information about the NIH Guidelines and OBA.

Basic laboratories –Biosafety Levels 1 and 2

For the purposes of this manual, the guidance and recommendations given as minimum requirements pertaining to laboratories of all biosafety levels are directed at microorganisms in Risk Groups 1–3. Although some of the precautions may appear to be unnecessary for some organisms in Risk Group 1, they are desirable for training purposes to promote good (i.e. safe) microbiological techniques (GMT). The guidelines for basic laboratories – Biosafety Levels 1 and 2 presented here are comprehensive and detailed, as they are fundamental to laboratories of all biosafety levels. The guidelines for containment laboratories – Biosafety Level 3 are modifications of and additions to these guidelines, designed for work with the more dangerous (hazardous) pathogens.

Code of practice

This code is a listing of the most essential laboratory practices and procedures that are basic to GMT. GMT are fundamental to laboratory safety. Specialized laboratory equipment is a supplement to but can never replace appropriate procedures. The most important concepts are listed below.

Access

1. The international biohazard warning symbol and sign (Figure 1) must be displayed on the doors of the rooms where microorganisms of Risk Group 2 or higher risk groups are handled.
2. Only authorized persons should be allowed to enter the laboratory working areas.
3. Laboratory doors should be kept closed.
4. Children should not be authorized or allowed to enter laboratory working areas.
5. Access to animal housing spaces should be specially authorized.
6. No animals should be admitted other than those involved in the work of the laboratory.

Personal protection

1. Laboratory coats, coveralls, gowns or uniforms must be worn at all times for work in the laboratory.
2. Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with blood, body fluids and other potentially infectious materials or infected animals. After use, gloves should be removed aseptically, and hands must then be washed.
3. Personnel must wash their hands after handling infectious materials and animals, and before they leave the laboratory working areas.
4. Safety glasses, face shields (visors) or other protective devices must be worn when it is necessary to protect the eyes and face from splashes, impacting objects and sources of artificial ultraviolet radiation.
5. It is prohibited to wear protective laboratory clothing outside the laboratory, e.g. in break rooms, coffee rooms, offices, libraries, staff rooms and toilets.
6. Open-toed footwear must not be worn in laboratories.
7. Eating, drinking, smoking, applying cosmetics and handling contact lenses is prohibited in the laboratory working areas.
8. Storing human foods or drinks anywhere in the laboratory working areas is prohibited.
9. Protective laboratory clothing that has been used in the laboratory must not be stored in the same lockers or cupboards as street clothing.

Procedures

1. Pipetting by mouth must be strictly forbidden.
2. Materials must not be placed in the mouth. Labels must not be licked.
3. All technical procedures should be performed in a way that minimizes the formation of aerosols and droplets.

4. The use of hypodermic needles and syringes should be limited. They must not be used as substitutes for pipetting devices or for any purpose other than parenteral injection or aspiration of fluids from laboratory animals.
5. All spills, accidents and overt or potential exposures to infectious materials must be reported to the laboratory supervisor. A written record of such accidents and incidents should be maintained.
6. A written procedure for the clean-up of all spills must be developed and followed.
7. Contaminated liquids must be decontaminated (chemically or physically) before discharge to the sanitary sewer. An effluent treatment system may be required, depending on the risk assessment for the agent(s) being handled.
8. Written documents that are expected to be removed from the laboratory need to be protected from contamination while in the laboratory.

Laboratory working areas

1. The laboratory should be kept neat, clean and free of materials that are not pertinent to the work.
2. Work surfaces must be decontaminated after any spill of potentially dangerous material and at the end of the working day.
3. All contaminated materials, specimens and cultures must be decontaminated before disposal or cleaning for reuse.
4. Packing and transportation must follow applicable national and/or international regulations.

Biosafety management

It is the responsibility of the laboratory director (the person who has immediate responsibility for the laboratory) to ensure the development, adoption and implementation of The Biosafety and Security Plan.

1. The laboratory director must ensure that regular training in laboratory safety is provided.
2. Personnel should be advised of special hazards and are required to read the safety or operations manual and follow standard practices and procedures. A copy of the safety or operations manual should be available in the laboratory.
3. There should be an arthropod and rodent control program.
4. Appropriate medical evaluation, surveillance and treatment should be provided for all personnel in case of need, and adequate medical records should be maintained.
5. Space and facilities should be provided for the safe handling and storage of solvents and compressed and liquefied gases.
6. Facilities for storing outer garments and personal items should be provided outside the laboratory working areas.
7. Facilities for eating and drinking and for should be provided outside the laboratory working areas.
8. Hand-washing basins should be provided in each laboratory room, preferably near the exit door.

9. Doors should have vision panels, appropriate fire ratings, and preferably be self-closing.
10. An autoclave or other means of decontamination should be available in appropriate proximity to the laboratory.
11. Safety systems should cover fire, electrical emergencies, emergency shower and eyewash facilities.
12. First-aid areas or rooms suitably equipped and readily accessible should be available.
13. A dependable supply of good quality water is essential. There should be no cross-connections between sources of laboratory and drinking-water supplies. An anti-backflow device should be fitted to protect the public water system.
14. There should be a reliable and adequate electricity supply and emergency lighting to permit safe exit. A stand-by generator is desirable for the support of essential equipment, such as incubators, biological safety cabinets, freezers, etc., and for the ventilation of animal cages.
15. There should be a reliable and adequate supply of gas. Good maintenance of the installation is mandatory.

Laboratory equipment

Together with good procedures and practices, the use of safety equipment will help to reduce risks when dealing with biosafety hazards. The laboratory director should, after consultation with the biosafety officer and safety committee ensure that adequate equipment is provided and that it is used properly. Equipment should be selected to take account of certain general principles, i.e. it should be:

1. Designed to prevent or limit contact between the operator and the infectious material
2. Constructed of materials that are impermeable to liquids, resistant to corrosion and meet structural requirements
3. Fabricated to be free of burrs, sharp edges and unguarded moving parts
4. Designed, constructed and installed to facilitate simple operation and provide for ease of maintenance, cleaning, decontamination and certification testing; glassware and other breakable materials should be avoided, whenever possible.

Essential biosafety equipment

1. Pipetting aids – to avoid mouth pipetting.
2. Biological safety cabinets, to be used whenever:
 - infectious materials are handled; such materials may be centrifuged in the open laboratory if sealed centrifuge safety cups are used and if they are loaded and unloaded in a biological safety cabinet
 - there is an increased risk of airborne infection
 - procedures with a high potential for producing aerosols are used; these may include centrifugation, grinding, blending, vigorous shaking or mixing, sonic disruption and opening of containers of infectious materials whose internal pressure may be different from the ambient pressure.
3. Plastic disposable transfer loops. Alternatively, electric transfer loop incinerators may be used inside the biological safety cabinet to reduce aerosol production.

4. Screw-capped tubes and bottles.
5. Autoclaves or other appropriate means to decontaminate infectious materials.
6. Plastic disposable serological pipettes, whenever available, to avoid glass.
7. Equipment such as autoclaves and biological safety cabinets must be validated with appropriate methods before being taken into use. Recertification should take place at regular intervals, according to the manufacturer's instructions.

Health and medical surveillance

IAVI is responsible for ensuring that there is adequate surveillance of the health of laboratory personnel. The objective of such surveillance is to monitor for occupationally acquired diseases. Appropriate activities to achieve these objectives are:

1. Provision of active or passive immunization where indicated
2. Facilitation of the early detection of laboratory-acquired infections
3. Laboratory personnel who may be highly susceptible to agents in use in the laboratory, such as pregnant or immunocompromised personnel, should seek the advice of Occupational Health physician and their personal physician to determine if they should work with specific agents.
4. Provision of effective personal protective equipment and procedures.

Guidelines for the surveillance of laboratory workers handling microorganisms at Biosafety Level 2

1. A pre-employment health check is required for all IAVI-DDL employees. This screening is performed at SUNY Downstate Student and Employee health center (440 Lenox Rd, Unit 1S) where blood will be collected for baseline status, and a medical history should be recorded, and a targeted occupational health assessment performed. Appointments may be scheduled by calling 718-270-1995.

2. In the event of a workplace exposure to potentially infectious agents:

If at SUNY, proceed to:

SUNY Downstate Student Health
440 Lenox Road, Apartment 1S

OR

SUNY Downstate ER (if after-hours)
If at DDL, proceed to: Lutheran Hospital ER
150 55th Street at 2nd Ave.

3. For possible exposures to nonhuman primate pathogens, including SIV, SHIV, or Herpes B, please refer to the detailed instructions in the IAVI SOP-0089 Post Exposure Plan for SIV or SHIV.

Transportation of Biohazardous (Infectious) Substances

Any materials which present a risk or potential risk to the health of humans, animals, or the environment are considered to be biohazardous (infectious) substances. These include bacteria, viruses, biologically active agents, certain types of recombinant DNA, as well as any samples which may contain these

substances such as tissue samples, blood, cells, or other potentially infectious material collected from infected animals. Such materials must be transported as described below (also described in SOP MC-0034 Transport of Biohazardous Material).

Transport of biohazardous substances within a research facility:

In the biosafety cabinet, decontaminate the vial or vessel containing biohazardous materials with 70% isopropanol or diluted coverage plus (SOP-0110 Preparation of Disinfectants). Place the vial or vessel into a sealable break-resistant container (e.g. Rubbermaid tote or Playmate-type cooler marked with a universal biohazard symbol and labeled with the name of the infectious agent) containing sufficient absorbent pads to absorb the entire contents of the primary container in case of breakage or leakage. Spray the transport container with 70% isopropanol and wipe down to ensure that the outside of the transport container is free of any biohazardous material. Transport the material to its intended location. Should a spill occur, clean following the instructions highlighted in SOP MC-0046 Cleaning Biological Spills.

Transport of biohazardous substances between buildings:

Materials will be contained within a leak-proof and labeled primary container such as a capped or sealed tube or vial. All primary containers will be sealed inside a biohazard-labeled plastic bag accompanied by enough absorbent material to contain the total volume of liquid, should all tubes/containers leak or break. The plastic bag will be placed within a unbreakable, leak-proof and puncture resistant secondary container labeled with the universal biohazard sign. The exterior of the secondary container should be decontaminated with 70% isopropanol.

Transport of biohazardous substances between sites using a courier or shipping service:

Shipping of hazardous materials is regulated by the U.S. Department of Transportation (DOT) and the International Air Transport Association (IATA). Infectious material must meet IATA standards and be packed in accordance with the World Health Organization (WHO) “Guidance on Regulations for the Transport of Infectious Substances 2009–2010” and Department of Transportation regulations in order to be transported via a licensed biological shipping or courier service.

http://www.who.int/csr/resources/publications/biosafety/WHO_HSE_EPR_2008_10.pdf?ua=1

A triple packaging system must be used as per the instructions provided by the WHO:

- Primary receptacle. A primary watertight, leak-proof receptacle containing the specimen. The receptacle is packaged with enough absorbent material to absorb all fluid in case of breakage.
- Secondary packaging. A second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material shall be used to absorb all fluid in case of breakage.
- Outer packaging. Secondary packagings are placed in outer shipping packagings with suitable cushioning material. Outer packagings protect their contents from outside influences, such as physical damage, while in transit. The smallest overall external dimension shall be 10x10 cm. Each completed package is required to be correctly marked, labelled [as per IATA guidelines] and accompanied with shipping documents.”

The outer container must be labeled with a sticker indicating that the contents are infectious substances, have the universal biohazard symbol, and list the infectious material(s) in the containers. If dry ice is

used, an additional sticker indicating that the package contains miscellaneous hazardous substances must also be used.

Annual Safety Training

Mandatory annual safety training will be held for all IAVI, Modern Meadow, and Avatar employees to be retrained on the following topics. If this training is not completed, access to IAVI DDL laboratory spaces will be retracted.

- General Safety
- Biosafety and Biological Exposure Plan
- Biosecurity
- Bloodborne Pathogens
- Chemical Safety

These trainings are required to be attended in person if possible. If an employee cannot attend the training, they must review the PowerPoint slides provided by the Safety Committee. All trainees must sign a sheet stating that they have received the training, and training must be completed within one month from the live training sessions or access to the IAVI-DDL laboratory spaces will be retracted.

The training materials for the above topics will be reviewed and revised on an annual basis by members of the IAVI Safety Committee. Any changes or new information that pertains to these issues will be addressed during monthly General Laboratory Meetings.

In addition to the annual safety training, all employees are required to read and sign-off on all Safety SOPs which are stored on Master Control.

New Employee Safety Training

All new IAVI DDL employees must complete the full complement of safety training listed below prior to gaining access to IAVI lab spaces. Entry will be denied until all training is completed.

A quiz must be completed following each training session, and the certificate following each training quiz must be printed and attached to a Safety Training Documentation Form and given to the Laboratory Coordinator.

Waste handling and disposal procedures

Waste is categorized as being either (i) non-hazardous, also referred to as “general waste”, (ii) hazardous non-sharp solid waste, (iii) hazardous sharp or potentially sharp waste, (iv) hazardous liquid waste, or (v) chemical waste. Disposal requirements are dictated by which category the solid waste falls under.

i) Non-hazardous waste should be disposed of as general waste, placed in black plastic bags.

ii) Hazardous non-sharp solid waste includes infectious or potentially infectious materials that present a risk or potential risk to the health of humans, animals, or the environment. Biohazardous waste may be material of bacterial, viral or animal origin, including items (labware, gloves, or other material) that have been in contact with them. It also may contain animal tissue or items that are contaminated with animal tissue or fluids. Wastes within this category must be disposed of following the guidelines set forth in SOP-MC-0042 Solid Biohazardous Waste Disposal.

Biohazard solid waste originated from working with viruses and infectious tissues and cells must be decontaminated by autoclaving or chemical decontamination before disposing in biohazard waste

cardboard boxes (SOP MC-0111 Operation and Maintenance of the Getinge Autoclave; SOP MC-0024 Chemical Decontamination of Biohazardous Material).

iii) Hazardous sharp or potentially sharp waste includes items designed to cut or penetrate, such as scalpel blades, suture needles, razor blades and hypodermic needles which have been contaminated with hazardous agents. This also includes broken potential sharps and broken glass which may be contaminated with hazardous agents.

It is important to include potential sharps in this category. These are items that can potentially break or shard or can perforate an autoclavable biohazard waste bag, such as vacutainer tubes (glass or plastic), blood or serum storage vials (glass or plastic), pipettes and pipette tips or glass slides.

Wastes within this category must be disposed of following the guidelines set forth in SOP-0042 Solid Biohazardous Waste Disposal.

iv) Liquid biohazardous waste includes waste such as animal or human blood or blood products, bacterial culture, or virus suspended in a liquid form must be disinfected before being disposed of. For handling of liquid biohazard waste see SOP-0039 Liquid Waste Disposal.

v) Chemical wastes are any liquid, solid or gaseous substances which are flammable, have toxic properties, can cause air and water pollution if released into the atmosphere, or produce adverse physiological reaction. Instructions for handling and disposing chemical wastes may be found in SOP-0091 Disposal of Empty Chemical Containers and SOP MC-XXXX Disposal of Chemical Waste.

Fire and electrical safety

Fire Safety:

To Report a Fire at the BAT:

- Activate the fire alarm pull
- Call the Fire Department to confirm fire location
- FDNY Unit 114; (718) 965-8314
- Notify the Fire Safety Officer, who will notify building management

To Report a Fire at the Incubator:

- Activate the fire alarm pull
- Call 911 to confirm fire location if David Norton is not available
- Notify the Fire Safety Officer
- Call David Norton at (917) 886-54

Before considering fighting a fire, call the fire department, confirm that the fire is small and not spreading, and confirm that you and others have a safe path to exit. Never fight a fire if the fire is spreading past the immediate area in which it began, if it could block your escape route, or if you are unsure about the proper operation of a fire extinguisher.

Once the fire alarm sounds or the safety warder has alerted you, proceed calmly to the nearest fire exit. Stay to the right when walking down the fire stairwell, exit the building, and proceed to the assembly area outside. In the event of a fire, do not use an elevator.

Electrical Safety:

Electrical hazards have the potential to cause fires. Faulty electrical equipment or the misuse of equipment may produce heat or sparks that serve as ignition sources. Space heaters are not permitted inside the laboratory. The use of temporary extension cords longer than six feet is not permitted, and the use of extension cords as permanent solutions is also not permitted in the lab. Extension cords and multi-plug strips may not be plugged into each other.

Survey for laboratory compliance

Safety audits will be performed once per month by two members of the Safety Committee. This audit will be performed on the 15th of each month +/- three business days and the findings of the audit will be disseminated via email to all members of the DDL and to the Avatar representative on the Safety Committee within 24 hours of the audit. Each laboratory space is assigned a "Responsible Party" whose role is to resolve any violations in a designated time period and to discuss with laboratory personnel any deficiencies noted during the audit so that they may be avoided in the future. Violations should be cleared and signed off on within one week of the audit. The documents are stored on Sharepoint.

If after five business days items have not been resolved and signed off on, the responsible party will be notified once more by the chairperson of the Safety Committee and will be given an additional five business days to resolve the violation. At the end of that five day period, the audit sheet will be locked off on Sharepoint and individuals will no longer be able to modify the audit document. Supervisors of the responsible parties who have not rectified violations will be notified.

Pest Control

Pest control program serves to eliminate insects and rodents without the use of conventional pesticides. It is established through Bell Environmental Services. Program ensures a safe and effective proactive approach to insect monitoring and rodent control and consists of regular bi-weekly visits by Bell's service personnel.

PART II- Laboratory Equipment

Biological safety cabinets

Biological safety cabinets (BSCs) are enclosed, ventilated workspace for safely working with contaminated materials. They are designed to protect the operator, the laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens. Aerosol particles are created by any activity that imparts energy into a liquid or semi liquid material, such as shaking, pouring, stirring or dropping liquid onto a surface or into another liquid. Other laboratory activities, such as streaking agar plates, inoculating cell culture flasks with a pipette, using a multichannel pipette to dispense liquid suspensions of infectious agents into microculture plates, homogenizing and vortexing infectious materials, and centrifugation of infectious liquids, or working with animals, can generate infectious aerosols. Aerosol particles of less than 5 μm in diameter are not visible to the naked eye. The laboratory worker is generally not aware that such particles are being generated and may be

inhaled or may cross-contaminate work surface materials. BSCs, when properly used, have been shown to be highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to aerosol exposures. Over the years the basic design of BSCs has undergone several modifications. A major change was the addition of a high-efficiency particulate air (HEPA) filter to the exhaust system. The HEPA filter traps 99.99% of particles as small 0.3 microns in size. This enables the HEPA filter to effectively trap all known infectious agents and ensure that only microbe-free exhaust air is discharged from the cabinet. A second design modification was to direct HEPA-filtered air over the work surface, providing protection of work surface materials from contamination. This feature is often referred to as product protection. These basic design concepts have led to the evolution of three classes of BSCs.

Class II biological safety cabinets

As the use of cell and tissue cultures for the propagation of viruses and other purposes grew, it was no longer considered satisfactory for unsterilized room air to pass over the work surface. The Class II BSC was designed not only to provide personnel protection but also to protect work surface materials from contaminated room air.

Class II BSCs, of which there are four types (A1, A2, B1 and B2), differ from Class I BSCs by allowing only air from a HEPA-filtered (sterile) supply to flow over the work surface. The Class II BSC can be used for working with infectious agents in BSL- 2 and 3.

Using biological safety cabinets in the laboratory Location

The velocity of air flowing through the front opening into a BSC is about 0.45 m/s. At this velocity the integrity of the directional air inflow is fragile and can be easily disrupted by air currents generated by people walking close to the BSC, open windows, air supply registers, and opening and shutting doors. Ideally, BSCs should be situated in a location remote from traffic and potentially disturbing air currents. Whenever possible a 30-cm clearance should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30–35 cm above the cabinet may be required to provide for accurate air velocity measurement across the exhaust filter and for exhaust filter changes.

Operators

If BSCs are not used properly, their protective benefits may be greatly diminished. Operators need to be careful to maintain the integrity of the front opening air inflow when moving their arms into and out of cabinets. Arms should be moved in and out slowly, perpendicular to the front opening. Manipulations of materials within BSCs should be delayed for about 1 min after placing hands and arms inside to allow the cabinet to adjust and to “air sweep” the surface of the hands and arms. The number of movements across the front opening should also be minimized by placing all necessary items into the cabinet before beginning manipulations.

Material placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items. Materials to be placed inside the cabinet should be surface-decontaminated with 70% alcohol. All materials should be placed as far back in the cabinet, towards the rear edge of the work surface as practical.

Bulky items, such as biohazard bags, discard pipette trays and suction collection flasks should be placed to one side of the interior of the cabinet. Active work should flow from clean to contaminated areas across the work surface.

The autoclavable biohazard collection bag and pipette collection tray should not be placed outside the cabinet. The frequent in-and-out movement needed to use these containers is disruptive to the integrity of the cabinet's air barrier and can compromise both personnel and product protection.

Operation and maintenance

Most BSCs are designed to permit operation 24 h/day, and investigators find that continuous operation helps to control the levels of dust and particulate materials in the laboratory. Class IIA1 and IIA2 BSCs exhausting to the room or connected by thimble connections to dedicated exhaust ducts can be turned off when not in use.

Other types such as IIB1 and IIB2 BSCs, which have hard-duct installations, must have airflow through them at all times to help maintain room air balance. Cabinets should be turned on at least 5 min before beginning work and after completion of work to allow the cabinet to "purge", i.e. to allow time for contaminated air to be removed from the cabinet environment.

All repairs made on BSCs should be made by a qualified technician. Any malfunction in the operation of the BSC should be reported and repaired before the BSC is used again.

Open flames

Open flames should be avoided in the near microbe-free environment created inside the BSC. They disrupt the airflow patterns and can be dangerous when volatile, flammable substances are also avoided. To sterilize bacteriological loops, microburners or electric "furnaces" are available and are preferable to open flames.

Spills

A copy of the laboratory's protocol for handling spills should be posted, read and understood by everyone who uses the laboratory (OP1.001). When a spill of biohazardous material occurs within a BSC, clean-up should begin immediately, while the cabinet continues to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent should be disinfected and/or autoclaved.

Certification

The functional operation and integrity of each BSC should be certified to national or international performance standards at the time of installation and regularly thereafter by qualified technicians, according to the manufacturer's instructions. Evaluation of the effectiveness of cabinet containment should include tests for cabinet integrity, HEPA filter leaks, down flow velocity profile, face velocity, negative pressure/ventilation rate, air-flow smoke pattern, and alarms and interlocks. Optional tests for electrical leaks, lighting intensity, ultraviolet light intensity, noise level and vibration may also be conducted. Special training, skills and equipment are required to perform these tests and it is highly recommended that they are undertaken by a qualified professional.

Cleaning and disinfection

All items within BSCs, including equipment, should be surface-decontaminated and removed from the cabinet when work is completed. The interior surfaces of BSCs should be decontaminated before and after each use.

The work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the work day, the final surface

decontamination should include a wipe-down of the work surface, the sides, back and interior of the glass. A solution of bleach or 70% alcohol should be used where effective for target organisms. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used. It is recommended that the cabinet is left running. If not, it should be run for 5 min in order to purge the atmosphere inside before it is switched off.

Decontamination

BSCs must be decontaminated before filter changes and before being moved. The most common decontamination method is by fumigation with formaldehyde gas. BSC decontamination should be performed by a qualified professional.

Personal protective equipment

Personal protective clothing should be worn whenever using a BSC. Laboratory coats are acceptable for work being performed at Biosafety Levels 1 and 2. Gloves should be pulled over the wrists of the gown rather than worn inside. Elasticized sleeves can be worn to protect the investigator's wrists. Safety glasses should be worn at all times while working in the BSC. Masks for protective inhalation may be required for some procedures.

Alarms

BSCs can be equipped with one of two kinds of alarm. Sash alarms are found only on cabinets with sliding sashes. The alarm signifies that the operator has moved the sash to an improper position, higher than recommended. Corrective action for this type of alarm is returning the sash to the proper position, marked on the sides of the BSC. Airflow alarms indicate a disruption in the cabinet's normal airflow pattern. This represents an immediate danger to the operator or product. When

an airflow alarm sounds, work should cease immediately and the laboratory supervisor should be notified. Manufacturer's instruction manuals should provide further details.

Safety equipment

As aerosols are important sources of infection, care should be taken to reduce the extent of their formation and dispersion. Hazardous aerosols can be generated by many laboratory operations, e.g. blending, mixing, grinding, shaking, stirring, sonicating and centrifuging of infectious materials. Even when safe equipment is used, it is best to carry out these operations in an approved biological safety cabinet whenever possible. The use of safety equipment is no assurance of protection unless the operator is trained and uses proper techniques. Equipment should be tested regularly to ensure its continued safe performance

Pipetting Aid

A pipetting aid must always be used for pipetting procedures. Aerosols can be generated when a liquid is dropped from a pipette on to a work surface, when cultures are mixed by alternate sucking and blowing, and when the last drop is blown out of a pipette. The inhalation of aerosols unavoidably generated during pipetting operations can be prevented by working in a biological safety cabinet. Pipetting aids should be selected with care. Their design and use should not create an additional infectious hazard and they should be easy to sterilize and clean. Plugged (aerosol-resistant) pipette tips should be used when manipulating microorganisms and cell cultures.

Pipettes with cracked or chipped suction ends should not be used as they damage the seating seals of pipetting aids and so create a hazard.

Homogenizers, shakers, blenders and sonicators

Only equipment designed for laboratory use should be used. Their construction minimizes or prevents aerosols release. Sonicators may release aerosols. They should be operated in biological safety cabinets or covered with shields during use. The shields and outsides of sonicators should be decontaminated after use.

Personal protective equipment and clothing

Personal protective equipment and clothing may act as a barrier to minimize the risk of exposure to aerosols, splashes and accidental inoculation. The clothing and equipment selected is dependent on the nature of the work performed. Protective clothing should be worn when working in the laboratory. Before leaving the laboratory, protective clothing should be removed, and hands should be washed.

Laboratory coats, gowns, coveralls, aprons

Laboratory coats should preferably be fully buttoned. However, long-sleeved, back-opening gowns or coveralls give better protection than laboratory coats and are preferred in microbiology laboratories and when working at the biological safety cabinet. Aprons may be worn over laboratory coats or gowns where necessary to give further protection against spillage of chemicals or biological materials such as blood or culture fluids. Laundering services should be provided at/near the facility.

Laboratory coats, gowns, coveralls, or aprons should not be worn outside the laboratory areas.

Goggles, safety spectacles, face shields

The choice of equipment to protect the eyes and face from splashes and impacting objects will depend on the activity performed. Prescription or plain eye glasses can be manufactured with special frames that allow lenses to be placed in frame from the front, using shatterproof material either curved or fitted with side shields (safety glasses). Safety spectacles do not provide for adequate splash protection even when side shields are worn with them. Goggles for splash and impact protection should be worn over normal prescription eye glasses.

NO contact lenses are allowed while working in the lab, since vapors from chemicals can be trapped between the eye and the lens and cause damage to the eye. Face shields (visors) are made of shatterproof plastic, fit over the face and are held in place by head straps or caps.

Goggles, safety spectacles, or face shields should not be worn outside the laboratory areas.

Respirators

Respiratory protection may be used when carrying out high-hazard procedures (e.g. cleaning up a spill of infectious material). The choice of respirator will depend on the type of hazard(s). Respirators are available with interchangeable filters for protection against gases, vapors, particulates and microorganisms. It is imperative that the filter is fitted in the correct type of respirator. To achieve optimal protection, respirators should be individually fitted to the operator's face and tested. Fully self-contained biological agents. Respirators should not be worn outside the laboratory areas.

Gloves

Contamination of hands may occur when laboratory procedures are performed. Hands are also vulnerable to “sharps” injuries. Disposable microbiologically approved latex, vinyl or nitrile surgical-type gloves are used widely for general laboratory work, and Gloves should be removed and hands thoroughly washed after handling infectious materials, working in a biological safety cabinet and before leaving the laboratory. Used disposable gloves should be discarded with infected laboratory wastes.

Allergic reactions such as dermatitis and immediate hypersensitivity have been observed with latex glove, with and without containing powder. Allergic responses to the powders have also been observed.

Part III- Safety Organization

The biosafety officer and biosafety committee

It is essential that each laboratory organization has a comprehensive safety policy, a safety manual, and supporting programs for their implementation. Laboratory safety is also the responsibility of all supervisors and laboratory employees, and individual workers are responsible for their own safety and that of their colleagues. Employees are expected to perform their work safely and should report any unsafe acts, conditions or incidents to their supervisor. Periodic safety audits by internal or external personnel are desirable.

Biosafety officer

Wherever possible a biosafety officer should be appointed to ensure that biosafety policies and programs are followed consistently throughout the laboratory. The biosafety officer executes these duties on behalf of the head of the institute or laboratory. In small units, the biosafety officer may be a microbiologist or a member of the technical staff, who may perform these duties on a defined part-time basis. Whatever the degree of involvement in biosafety, the person designated should possess the professional competence necessary to suggest, review and approve specific activities that follow appropriate biocontainment and biosafety procedures. The biosafety officer should apply relevant national and international rules, regulations and guidelines, as well as assist the laboratory in developing standard operating procedures. The person appointed must have a technical background in microbiology, biochemistry and basic physical and biological sciences. Knowledge of laboratory and clinical practices and safety, including containment equipment, and engineering principles relevant to the design, operation and maintenance of facilities is highly desirable. The biosafety officer should also be able to communicate effectively with administrative, technical and support personnel.

The activities of the biosafety officer should include the following:

1. Biosafety, biosecurity and technical compliance consultations.
2. Periodic internal biosafety audits on technical methods, procedures and protocols, biological agents, materials and equipment.
3. Discussions of violation of biosafety protocols or procedures with the appropriate persons.
4. Verification that all staff have received appropriate biosafety training.
5. Provision of continuing education in biosafety.

6. Investigation of incidents involving the possible escape of potentially infectious or toxic material and reporting of findings and recommendations to the laboratory director and biosafety committee.
7. Coordination with medical staff regarding possible laboratory-acquired infections.
8. Ensuring appropriate decontamination following spills or other incidents involving infectious material(s).
9. Ensuring proper waste management.
10. Ensuring appropriate decontamination of any apparatus prior to repair or servicing.
11. Maintaining awareness of community attitudes regarding health and environmental considerations.
12. Establishment of appropriate procedures for import/export of pathogenic materials.
13. Reviewing the biosafety aspects of all plans, protocols and operating procedures.
14. Institution of a system to deal with emergencies.

Biosafety committee

A biosafety committee should be constituted to develop institutional biosafety policies and codes of practice. Other functions of the committee may include risk assessments, formulation of new safety policies and arbitration in disputes over safety matters. The membership of the biosafety committee should reflect the diverse occupational areas of the organization as well as its scientific expertise. The composition of a basic biosafety committee may include:

1. Biosafety officer
2. Scientists
3. Medical personnel
4. Representatives of technical staff
5. Representatives of laboratory management.

The biosafety committee should seek advice from different departmental and specialist safety officers and may at times require assistance from independent experts in various associated fields, local authorities and national regulatory bodies.

Annex 3

Sample Post Exposure Plan for the Occupational Exposure to Simian Immunodeficiency Virus (Siv) or Simian-Human Immunodeficiency Virus (Shiv)

Purpose

The purpose of this protocol is to describe how to respond to an accidental exposure to Simian Immunodeficiency Virus (SIV) or Simian-Human Immunodeficiency Virus (SHIV).

1 SCOPE

Individuals working with SIV or SHIV in the laboratory, individuals working with non-human primates (NHPs) who have been experimentally challenged with SIV or SHIV, or individuals working with tissues/blood products/mucosal swabs/fluids collected from experimentally challenged NHPs must adhere to these guidelines.

Any exposure to NHP blood, blood products, mucosal samples, or tissues, regardless of whether the exposure involves SIV or SHIV challenged or infected NHPs, requires adherence to *SOP-0041 Exposure to Infectious Agents*, which details procedures to responding to the potential of Herpes B virus exposure.

2 DEFINITIONS

- 3.1 Simian Immunodeficiency Virus (SIV) – a lentivirus whose natural host is Old World NHPs. SIV causes AIDS-like symptoms in some species of NHPs but is not fully understood if it is non-pathogenic in humans. SIV from chimpanzees, gorillas, and sooty mangabey monkeys crossed into humans to become the precursors of HIV-1 and HIV-2. *SIV is stored but not currently being used at the DDL/EDL; No human vaccine available*
- 3.2 Simian-Human Immunodeficiency Virus (SHIV) – a laboratory engineered virus which consists of tat, rev, vpu, and env genes from both HIV and all other genes from SIV. Because of its combination of SIV and HIV genes, SHIV can cause AIDS-like symptoms in both NHPs and humans. *SHIV is stored and currently being used at the DDL/EDL; No human vaccine available*
- 3.3 EDTA-Coated Tubes – blood collection tubes coated with EDTA as an anticoagulant are used when collecting blood from an individual who has been exposed to SIV or SHIV

3 RESPONSIBILITIES

- 4.1 IAVI research staff must adhere to the guidelines set forth by this SOP following an accidental exposure to SIV/SHIV

- 4.2 IAVI research staff must adhere to the guidelines set in this SOP following an accidental exposure to tissues or fluids from an NHP that has been experimentally infected with SIV or SHIV
- 4.3 IAVI's Associate Director of Vector Immunobiology is responsible for ensuring the immediate shipment and testing of specimens from the exposed individual
- 4.4 The laboratory of Bill Switzer at the CDC will be performing the anonymous testing and archiving of samples provided by the IAVI Associate Director of Vector Immunobiology

4 MATERIALS AND EQUIPMENT

5.1 Materials

- 5.1.1 Disposable gloves (VWR Catalog # MF300S, MF300M, MF300L or equivalent), face shield or safety glasses, lab coat
- 5.1.2 BD™ E-Z Scrub™ Surgical Scrub Brush (Fisher Scientific Catalog # 22-265-650)
- 5.1.3 Timer

5.2 EQUIPMENT

- 5.2.1 Eyewash station/ sink

5 PROCEDURE

5.1 PROCESS FLOW

Not Applicable

5.2 OVERVIEW

- 6.2.1 When an exposure to SIV or SHIV occurs, the first priority is to flush or wash the site of exposure
- 6.2.2 When working with SIV or SHIV virus stock or tissues or body fluids from infected NHPs, routes of exposure may be percutaneous (via a cut, needle-stick, abrasion, open wound, or otherwise non-intact skin) or mucosal (via a splash to the eyes, mouth, or nose)

- 6.2.3 When working with SIV or SHIV challenged NHPs, routes of exposure may be percutaneous (via a needle-stick, scratch, or bite from a SIV or SHIV challenged NHP) or mucosal (via a splash)

5.3 FIRST AID RESPONSE TO A PERCUTANEOUS EXPOSURE

- 5.3.1 Immediately cleanse the exposed site under running water while scrubbing with a betadine Surgi-Prep brush for 15 minutes (use timer to ensure a full 15 minute scrub and rinse)

5.4 FIRST AID RESPONSE TO A MUCOSAL EXPOSURE

- 5.4.1 Immediately irrigate the site with running water for 15 minutes, using an eyewash station if the exposure was to the eyes (use timer to ensure a full 15 minute flush)

5.5 COLLECTION AND SHIPMENT OF WHOLE BLOOD SPECIMENS TO THE CDC:

- 5.5.1 If at **Brooklyn Army Terminal**, proceed to:
Lutheran Hospital ER
150 55th Street at 2nd Ave.
If at **SUNY Downstate**, proceed to:
SUNY Downstate Student Health
440 Lenox Road, Apartment 1S

OR

SUNY Downstate ER (if after-hours)

- 6.5.2 **Take a copy of this SOP with you and tell the physician that you have had a potential exposure to SIV or SHIV.**

NOTE: Any exposure to NHP blood, blood products, mucosal samples, or tissues, regardless of whether the exposure involves SIV or SHIV challenged or infected NHPs, requires adherence to *SOP-0041 Exposure to Infectious Agents*, which details procedures to responding to the potential of Herpes B virus exposure.

- 6.5.3 **Request that 20 mL of EDTA-treated blood be collected and inform the physician that you will take your samples with you and label and ship them to the CDC yourself**

- 6.5.4 Label the specimen tubes and paperwork in a manner that does not include personally identifiable information. Specifically, label the tubes with “IAVT”,

the date the blood was collected, and how much time has passed since the exposure (i.e. Day 0, Week 3, Week 6, etc.)

- 6.5.5 Ship the package at **room temperature** to the CDC Serum Bank using an overnight, next morning delivery service to the address below:

CDC Serum Bank (STAT)
Centers for Disease Control and Prevention
1600 Clifton Road NE
Atlanta, GA 30329
“Attention: Project 139”

- 6.5.6 Provide email notification with package tracking number to **Bill Switzer** (CDC Diagnostics and Incidence Team Lead at the Laboratory Branch, Division of HIV/AIDS Prevention), bis3@cdc.gov

The email notification should include a scanned copy of the shipping manifest and details of the specific exposure in an email to Bill Switzer.

It should also detail when, where, and how the exposure occurred, the strain of SIV or SHIV involved, animal species (if the exposure was through a challenged/infected laboratory animal or their tissues), occupation of exposed individual, and any antiretroviral treatment the individual is receiving.

- 6.5.7 Exposed individual must fill out an IAVI Accident and Biohazardous Exposure Incident Report which may be found on the IAVI Sharepoint:
<https://iavi.sharepoint.com/offices/DDL/SitePages/Biosafety.aspx>

5.6 POST EXPOSURE PROPHYLAXIS SHOULD BE DISCUSSED WITH THE PHYSICIAN

- 5.6.1 Post Exposure Prophylaxis (PEP) regimens for SIV or SHIV recommended by the CDC clinic:

HIV PEP Regimen

Raltegravir (Isentress; RAL) 400 mg PO twice daily

Plus

Truvada, 1 PO once daily

(Tenofovir DF [Viread; TDF] 300 mg _ emtricitabine [Emtriva; FTC] 200 mg)

5.7 CONTINUED SURVEILLANCE

- 5.7.1 Post Exposure, the exposed individual should be re-tested at 3 weeks, 6 weeks, 3 months, and 6 months

6 REFERENCES

- Occupational Health and Safety in the Care and Use of Nonhuman Primates

- <http://www.nap.edu/catalog/10713.html> for free pdf download (pages 28-29 focus on SIV)
- *SOP-0041 Exposure to Infectious Agents*

7 FORMS, RECORDS, TOOLS

- IAVI Accident and Biohazardous Exposure Incident Report
<https://iavi.sharepoint.com/offices/DDL/SitePages/Biosafety.aspx>

ANNEX 4.

IAVI-DDL Laboratory Safety Audit Checklist

Laboratory signage
Equipment signage: No food/drink on refrigerators and freezers; biohazard on centrifuges used for infectious substances
Entry requirement signage- Guidelines posted at entry and followed:
Biohazard sign posted on laboratory door (sign contains biohazard symbol, PPE required, agents in use/storage, emergency contact info)
Access limited and restricted to authorized personnel
Information on sign accurate and current- to be revised and re-posted annually
Sign legible and not defaced
Laboratory storage and organization
Designed for easy cleaning
All shelves secured with heavy items stored on lower shelves
Minimum 1 foot clearance between items being stored on shelves, and ceiling
Adequate illumination provided
Adequate storage space available and appropriately used without clutter
Gas cylinders
All cylinders secured
Caps on reserve cylinders
Asphyxiating and hazardous gases only in ventilated rooms
Fire Extinguishers
Extinguisher must be visible, unobstructed, with clear signage
Extinguisher must be mounted securely to wall
If gauge, arrow must point to green
Ensure no obvious physical damage, corrosion, leakage, or clogged nozzle
Must be checked and tag signed off on monthly basis
Refrigerators/freezers/cold rooms
No food or drink for human consumption present; Refrigerators and freezers labeled with “No food/drink” signage
Flammables (excluding small secondary bottles) stored in explosion-proof/-safe units
Labeled externally if containing carcinogens, and/or biohazards
Electrical equipment
Extension cords secured and not presenting a tripping hazard; No daisy-chaining of extension cords
Outlets earthed/grounded and with proper polarity
No cords or connections by sinks, under showers, by water sources, etc.
Equipment should not have frayed or damaged wiring
Outlets or electrical strip must not be overloaded
Power strips mounted off the floor
Grounds present on electrical cords
No portable space heaters permitted in labs
Personal protective equipment
Personal protective equipment (PPE) available and in good condition- lab coats, disposable gloves, eye protection
Occupants properly attired in long pants and close toed shoes
Required PPE being worn in the laboratory; Not worn in office areas
Personal protective equipment available for cryogenic storage
Waste management
Proper waste disposal (biohazard bins, chemical decontamination, autoclave decontamination)
Wastes segregated in proper containers (sharps, biohazard, broken glass, chemical wastes, etc.)

Sharps containers used and disposed of properly
No trash on floor
Waste vessels filled no greater than $\frac{3}{4}$ full
Infectious waste containers used and labeled with name of material, date, and hazards
Containers filled no greater than $\frac{3}{4}$ full
Containers securely closed and sealed upon reaching $\frac{3}{4}$ full
Culture stocks and other liquid waste decontaminated before disposal
Biological Safety Cabinets
Date Last Certification Present (annual)
BSC not compromised by room air or location
BSC used when there is potential for creating aerosols; Work with infectious material performed within BSC when possible
Liquid Waste Vacuum Flasks
Vacuum flask must contain $\frac{1}{5}$ volume of concentrated bleach
0.22 μ m vacuum filter and overflow flask must be used to protect the house vacuum system from contamination
Exposure Kits, eyewashes, safety showers
Eyewash available in laboratory and flushed weekly
Safety shower available and flushed monthly
First aid kits, chemical spill kits, biological spill kits, and NHP exposure kits available with non-expired contents
Freezer Biosecurity
All freezers containing infectious agents, USDA regulated agents must be locked at all times
Contact information must be posted on storage freezers
Labeling on freezer must include agents stored within