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Experiment Safety Review Form
Review Number: PS-ESR-1-166-2012

PRINCIPAL INVESTIGATOR: Vivian Stojanoff
GROUP: Life & Env Sciences
EXT: 8375 **E-MAIL: stojanof@bnl.gov** **LIFE NUMBER: 20327**

Project Title: Protein Crystal Preparation Lab	
Location(s): 0725	
Area(s): 0725-FIRST-1-166	
Proposed Start Date and Duration: 4/1/2012 - 1 years	

SIGNATURES:

Principal Investigator: Vivian Stojanoff	**Date: 4/6/2012**
Experiment Review Coordinator: Lori Stiegler	**Date: 4/6/2012**
Co-PI or Alternate Contact (s): Edwin Lazo	**Date:**
Reviewer: Christopher Weilandics	**Date: 3/21/2012**
Reviewer: Frank Zafonte	**Date: 3/22/2012**
Reviewer: Mary Anne Corwin	**Date:**
Reviewer: Deborah Bauer	**Date: 4/9/2012**
Reviewer: Brian Heneveld	**Date: 3/22/2012**
Approval: Lori Stiegler	**Date: 4/6/2012**
Review/Approval (ERC) Comments: 04/06/2012 1:15 PM	
Walkthrough Signature:	**Date:**
Expiration Date (max 1 yr.): 4/6/2013	
FUA Change Required? No	
Fire Rescue Run Card Changes Required? No	
Has a NEPA Review been Performed for this Project? No	
Required Approvals (i.e., IACUC, IBC, etc.):	
Project Termination Acceptance Signature: **Comments:**	**Date:**

I. Define the Scope of the Work

A. Description

1. Protein Crystal growth requires the preparation of protein solutions and buffers:

Lyophilized proteins are dissolved in a buffer (e. g. sodium chloride, ammonium sulfate, buffer pH 4.0 to 8.0). Proteins and buffers are prepared by weighing the solid components on a balance and dissolving in distilled water. Afterwards, the pH-value is checked by the means of a pH Meter (Fisher Scientific). After each use the balance gets cleaned so that there are no residues of protein left which may affect the next user. The proteins are of different nature (some are enzymes, some are not) and from different sources (animals, plants, microorganisms, human origin) and do not pose any biological hazard. Buffers and proteins are setup in standard crystallization plates that are commercially available. Periodically hydrochloric acid and sodium hydroxide are used in preparing buffer solutions. Various organic solvents (ethanol, acetone, isopropanol and methanol) are used for cleaning glassware and crystallization trays.

In some instances premixed buffers (CMS registered) commercially available from vendors such as Hampton Research, Qiagen, Molecular Dimensions are used as additives. These buffers may contain traces of heavy metals (Ba, Hg, Se, etc.) and some contain toxic materials (arsenic and cyanide compounds). These buffers are disposed as hazardous waste.

Storage and use of cryogens: There are 2 - 30 liters Dewars which are typically filled with liquid nitrogen for protein crystal storage. Filling of these Dewars is accomplished by periodically adding LN2 from a non pressurized 5 liter Dewar. Small open Dewars of approximately one liter may also be used for manipulation of protein crystals.

Chloroform is occasionally used for characterization of membrane proteins. Chloroform handling is performed in a hood labeled as a carcinogen area.

Typical equipment in the lab which may be used by trained users and staff includes:

- AKTA purification unit
- centrifuge (user interlock)
- sterilizer (user interlock)
- glass washing machine
- PHAST system for gel
- satellite waste accumulation area (only if trained)
- ladders (only if trained)

Users may use the following equipment which pose no particular hazards:

- incubators
- pipettes
- microscopes
- pH meters
- Millipore water purifier
- balances
- small centrifuge
- vortex
- UV spectrometer
- laminar flow bench

Equipment manuals or procedures that are controlled documents:

N/A

B. Human Performance Factors

The largest risk comes from the transferring of small volumes of liquid nitrogen to open Dewars.

C. Waste Minimization/Pollution Prevention

Waste minimization practices include only using the smallest appropriate amount of chemicals possible to perform the task at hand. Also frequent use of commercially available plates and solutions reduces the need for preparing larger batches of buffers and chemical solutions. When necessary to prepare a special solution only the amount expected to be used is prepared.

D. Materials Used /Waste Generated

Materials Used	Disposal Method	Amount per Use	Amount per Year	Comments
Buffer solutions containing toxic compounds	Hazardous	20.00 ml	1.00 ltr	
Corrosive waste (HCl, NaOH)	Hazardous	20.00 ltr	0.50 ltr	
Solvents	Hazardous	20.00 ml	0.50 ltr	
Chloroform	Hazardous	20.00 ml	100.00 ml	Carcinogen
Pump oil	Industrial	1.00 ltr	1.00 ltr	

II. Identify and Analyze Hazards Associated with the Work

The following hazards were identified:

Physical Hazards:

- Sharps (non medical)
- Cryogens (any substance or device capable of producing temperatures <= 170K)
- Portable Ladders
- Autoclaves or high temperature ovens
- Flammable liquids

Chemical Hazards:

- Chemicals, Hazardous (General)
- Carcinogens (Area: 0725-FIRST-1-166)
- Corrosives
- Flammable liquids (Area: 0725-FIRST-1-166)
- Toxic metals (e.g., As, Ba, Be, Cd, Cr, Hg, Pb, Se, Ag) (Area: 0725-FIRST-1-166)

Ionizing and Non-ionizing Radiation Hazards:

- Ultraviolet sources >1 W (Area: 0725-FIRST-1-166)

Biological Hazards:

- None

Offsite Work:

- None

Other Issues (Security, Notifications, Community, etc.):

- None

Significant Environmental Aspects

- Any amount of hazardous waste generation (Area: 0725-FIRST-1-166)
- Any amount of industrial waste generation (e.g., oils, vacuum pump oil)

III. Develop and Implement Hazard Controls and Assess Risk

A. Physical Hazards, Tasks and Controls

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Sharps (non medical)** **Default Controls:** Sharps including needles, razor blades and syringes (plastic and glass) must be disposed of in sturdy, rigid, sharps containers. Sharps containers cannot be more than 2/3 full. **Task Specific Info:** Wear cut resistant gloves when possible to prevent hand injury.	**Negligible (0-20)**
Hazard: **Cryogens (any substance or device capable of producing temperatures <= 170K)** **Default Controls:** General Requirements: • Evaluate location oxygen deficiency • Store/transport only in approved containers (i.e. DOT/ASME or BNL LESHC) • Never pour from above chest level • PPE: Long Sleeve Shirt (or Lab Coat), long pants (or skirt covering ankles) and closed shoes Pressurized transfer to open (vented) container; Or-Pouring > 5 liter volumes of LN2 between open containers: • Face shield along with either Safety Glasses (w/side shields) or Goggles • Gloves (Cryo or Heavy Leather) Pouring small (5 liters or less) volumes of LN2 between open containers: • Safety Goggles (face shield recommended if possible) • Gloves (Cryo or Heavy Leather) Work with samples immersed in LN2 in small (~1 liter) dewars: • Use Tongs (tools) to manipulate/handle cryogenic samples (do not touch with gloves)• Use insulated non-absorbent gloves with dexterity (cotton/nylon gloves under disposable nitrile gloves) • Safety Goggles	**Negligible (0-20)**
Hazard: **Portable Ladders** **Default Controls:** General Guidance for all devices: • Surfaces must be free from oil, grease and slippery substances • Stepping surface, including rungs, must be skid resistant • All feet or bottom surfaces must be skid resistant (i.e. rubber feet or ring) • All ladders, step stools and ladder stands must be removed from service immediately if any defects are found Step Stools and Ladder Stands: • The wheels must retract and rubber feet engage when stepped on Ladders, Stepladders, and Ladder Stands: • Ladders must be rated for industrial or commercial use (Type I or II). No Type III household ladders are to be used at NSLS. • All rungs and side rails must be in good condition with no cracks, or defects, such as bent supports • Ladders must be stable, with tight joints between the rungs and side rails • Stepladders must be equipped with a metal spreader or locking device of sufficient size and strength to securely hold the front and back sections in the open position Comply with Subject Area “Fall Protection”	**Acceptable (21-40)**
Hazard: **Autoclaves or high temperature ovens** **Default Controls:** • All liquids must be put in secondary containment with a small amount of water in bottom of	**Negligible (0-20)**

secondary containment.
• Use only Type I borosilicate glass bottles. Do not use ordinary glass bottles or any container not designated for autoclave use.
• Do not use plastic containers (polyethylene) that will melt or distort during autoclaving.
• Autoclavable plastic bags containing waste should be placed in secondary containers to retain liquid waste that may accidentally leak through the bags.
• Loosen screw caps on bottles and tubes of liquids before autoclaving. Never put sealed containers in an autoclave. They can explode. Large bottles with narrow necks may also explode if filled too full of liquid.
• Never put solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.), or radioactive materials in an autoclave.
• Prevent steam burns and shattered glassware by making sure that the pressure in the autoclave chamber is at zero before opening the door at the end of a cycle. Slowly crack open the autoclave door and allow the steam to escape gradually. Keep face away from door as it opens.
• Allow items to cool for 10 minutes before removing them from the autoclave. Liquids removed too soon may boil up and out of container, burning operator.
• PPE: Wear heat resistant gloves and eye protection, especially when unloading the autoclave.

Task Specific Info:

Autoclave is interlocked to prevent access while hot.

Hazard:	**Flammable liquids** **Default Controls:** As for chemicals, plus Store large quantities in Flam. cabinets as required	**Negligible (0-20)**

B. Chemical Hazards, Tasks and Controls

	Hazard, Default Controls, Task Specific Info	Risk Level
Hazard:	**Chemicals, Hazardous (General)** **Default Controls:** • All operations with large (>250ml, health hazard 3) quantities of hazardous chemicals (pouring, mixing, evaporation, etc) in hood, or use snorkel when hood is impractical • Register Commercial Chemicals in CMS • Work alone after hours only if permitted by supervisor or ESR • Identify containers so contents are identifiable unless being actively used (ex. 1 shift) • Food, beverage, smoking, and cosmetics are prohibited • Handle glassware properly: no mouth suction, no drinking from labware. **Task Specific Info:** Put plastic chain across doorway when using chemicals to indicate Lab Coats Required.	**Negligible (0-20)**
Hazard:	**Carcinogens** **Default Controls:** Use hazardous chemicals controls plus: Establish and post a "designated area" where used Note location on fire run card and hazard info. placard. Exposure monitoring to assure that action levels not exceeded Establish procedures for work area cleanup and decontamination **Task Specific Info:** Use only in fume hood. Lab coat, safety glasses and nitrile gloves	**Negligible (0-20)**
Hazard:	**Corrosives**	**Negligible (0-20)**

	Default Controls: Use hazardous chemicals controls plus: - Unobstructed access to Emergency Eyewash and Shower **Task Specific Info:** Keep lab door open when working with corrosives for access to eyewash/shower. Nitrile gloves, safety glasses, lab coat	
Hazard:	**Flammable liquids** **Default Controls:** Use hazardous chemicals controls. Review large quantity storage with Fire Protection Engineer Note location on fire run card and hazard info. placard for storage of solids, greater than 40 pounds; liquids, greater than 5 gallons; gases, greater than 10 pounds	**Negligible (0-20)**
Hazard:	**Toxic metals (e.g., As, Ba, Be, Cd, Cr, Hg, Pb, Se, Ag)** **Default Controls:** As for chemicals, plus need for SHSD and OMC monitoring and surveillance must be evaluated BURF for Beryllium operation **Task Specific Info:** Lab coat, eye protection, and gloves required when performing cell culture.	**Negligible (0-20)**

C. Environmental Hazards, Tasks and Controls (include on/off site transportation and products/services)

	Hazard, Default Controls, Task Specific Info	Risk Level
Hazard:	**Any amount of hazardous waste generation** **Default Controls:** Engineering Controls • Waste will be accumulated in chemically compatible containers that appropriately contain/protect the waste. • Waste containers will be closed in a tray (secondary containment) in the Satellite Accumulation Area (SAA). Administrative Controls • All hazardous waste containers will have a (red) “Hazardous Waste Label” that has the generator’s name and the chemical contents (trade name/formula not acceptable). • All waste will be accumulated in closed containers and kept in an established and posted SAA until ready for transfer to the 90-Day Haz Waste Area for pick-up by Waste Management. • For pick-up by Waste management, complete the Nonradioactive Haz Waste Control Form and consult the 90-Day Area Manager to gain access/transfer the waste to the 90Day Area. Training: Hazardous Waste Gen. (HP-RCRIGEN3). PPE: When handling waste materials follow PPE requirements specified for the specific materials. Comply with the SBMS Subject Area: “Hazardous Waste Management”.	**Negligible (0-20)**
Hazard:	**Any amount of industrial waste generation (e.g., oils, vacuum pump oil)** **Default Controls:** Engineering Controls • Store only compatible wastes together, in suitable containers. • Provide secondary containment for liquid wastes if potential for environmental release exists.	**Negligible (0-20)**

• Keep containers closed and secured unless adding waste to container. Administrative Controls • Use a green industrial waste label, with generator's name and chemical contents (trade name/formula NOT acceptable). Label oils "Used Oil". • When full, complete and submit a WCF for pick up. The waste may be stored in the 90-day area. Training: Hazardous Waste Generator (HP-RCRIGEN3) Comply with the SBMS Subject Area: "Industrial Waste".	

D. Radiation Hazards, Tasks and Controls

Hazard, Default Controls, Task Specific Info	Risk Level
Hazard: **Ultraviolet sources >1 W** **Default Controls:** Task and source specific. Evaluate below. **Task Specific Info:** Low wattage bulb, commercial unit with built-in user protection	**Negligible (0-20)**

E. Biological Hazards, Tasks and Controls
None

F. Offsite Work Hazards, Tasks and Controls
None

G. Other Issues (Security, Notifications to Other Organizations, Community Involvement, etc.)
None

H. Recommended Exposure Monitoring

- None

Description or comments:

I. EPHA Determination

Chemical Name	Quantity (lbs, gal)	Location (Bldg/Room#)

IV. Perform Work Within Controls

A. Recommended Training and Medical Surveillance Summary

- NSLS R2A2 for Lab Steward (LS-R2A2-LAB-STWD)
- Laboratory Standard (HP-IND-220)
- Cryogen Safety (HP-OSH-025)
- Hazardous Waste Generator (HP-RCRIGEN3)
- Portable Ladder Safety (TQ-LADDER)

B. Personnel Training, Qualification, and Authorization List

Employee/Guest Name	Life/Guest#	Dept	Required Training Course(s)	Signed
Vivian Stojanoff	20327	PS	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Laboratory Standard (HP-IND-220) [EXPIRED: 5/13/2012] Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 9/30/2012]	4/6/2012 12:00:00 AM

			Portable Ladder Safety (TQ-LADDER) [EXPIRES: NEVER]	
Jean Jakoncic	23409	PS	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Laboratory Standard (HP-IND-220) [EXPIRES: 3/1/2014] Portable Ladder Safety (TQ-LADDER) [EXPIRES: NEVER]	
Edwin Lazo	24406	PS	Cryogen Safety (HP-OSH-025) [EXPIRES: NEVER] Laboratory Standard (HP-IND-220) [EXPIRES: 5/27/2013] Hazardous Waste Generator (HP-RCRIGEN3) [EXPIRES: 3/19/2013] Portable Ladder Safety (TQ-LADDER) [EXPIRES: NEVER] NSLS R2A2 for Lab Steward (LS-R2A2-LAB-STWD) [UNASSIGNED: EXPIRES: 4/3/2013]	

C. Emergency Procedures

Spill control supplies are in the hallway outside the lab. In case any acid or bases are spilled into the eye go to the eye showers outside the lab and rinse thoroughly. After purging the eyes go to the OMC or call 2222 or 911. Follow the buildng 725 Local Emergency Plan.

D. Transportation

None

E. Logistical Interactions

None

F. Termination/Decommissioning

All equipment and chemicals will be transferred or dispositioned when the lab is decommissioned.

V. Provide Feedback

All work has been accomplished as expected.

VI. Attachments

Attached Files: