NANO & PICO CHARACTERIZATION LAB
California NanoSystems Institute
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BIOSAFETY for Rooms B123, B133 &B139

PURPOSE

The purpose of this document is to specify the procedures used to handle materials provided by other Laboratories outside of the Nano and Pico Characterization (NPC) Laboratory for the purpose characterization by scanning probe microscopy (SPM) and related methods. This SOP does not intent to supersede the SOP of the original PI's laboratory which supplied the samples, therefore this SOP is in addition to the original PI's SOP.

Prior to gaining access, the respective laboratories are required to indicate any and all biohazards emanating from their materials and have SOP's, IBC, IRB and/or ARC approvals in place under which the experiments will be conducted. All users must be properly trained by EH&S (see Safety Training Requirements below) and documented for proposed experiments on the PI's current IBC protocol. External users will need to initially sign a lab specific user agreement and a Core Facility User Agreement to certify that individuals are properly trained by EH&S and documented for proposed experiments on the PI's current IBC protocol (including other needed approvals i.e. IRB, ARC, Radiation Safety).

For non-UCLA users, they would need to submit a letter to UCLA IBC disclosing exactly what materials they are bringing into the lab and documentation that their institution's IBC has approved this work. If their institution does not have its own IBC, then the UCLA IBC may consider reviewing the IBC application and doing a full risk assessment of the project. External users must also attend required EH&S lab safety trainings or equivalents at their respective institution. Please see safety training requirements below. Responsibility for providing, arranging and documenting the appropriate training of users is the responsibility of their respective PI, not the NPC Laboratory.

For individuals requesting facility use, NPC staff will conduct appropriate facility-specific training along with a lab safety orientation. NPC will have a record of users' NPC-specific trainings. Only when training is complete will researchers be given scan access to the facility. Admittance to the NPC facility of persons other than registered users is strictly prohibited at all times, unless accompanied by NPC staff.

Given the fact that the works with over 70 PI's on campus, a common standard for handling has to be set to guarantee biosafety. This document will set this standard.

SCOPE

This SOP applies to any work carried out in the NPC laboratory that is not expressively defined as BSL-1.

RESPONSIBILITIES

The Principal Investigator is responsible for reviewing this SOP annually and updating this document as needed — as well as any and all other applicable documents.

All research personnel are responsible for reading and complying with the provisions herein.

SAFETY TRAINING REQUIREMENTS

In addition to receiving laboratory specific training for handling any samples, all research personnel handling biological samples must take the following EH&S safety training requirements.

A. Biosafety Cabinet (once)
B. Biosafety A,B,C's – Biosafety Level 2 (at least every 3 years)
C. Medical Waste Management (at least every 3 years)
D. Hazardous Chemical Waste Training (once), if this study involves hazardous chemicals
E. Blood Borne Pathogens

For external users, they must also attend the UCLA EH&S safety trainings above or equivalents at their own institution. If their respective institution does not have their own EH&S committee, they must take the EH&S safety trainings at UCLA.

Responsibility for providing, arranging and documenting the appropriate training of Users is the responsibility of their respective PI, not the NPC Laboratory. The NPC Director is responsible for providing or arranging the appropriate training of lab staff/personnel as well as maintaining their training records.

NPC LABORATORY SPECIFIC TRAINING

All users of the NPC Laboratories are required to receive a lab-specific safety orientation. A copy of a diagram of the locations of all the specific safety features is posted in Rooms B23 and B139 and is also available to users on request. Users must date and sign the NPC Safety Orientation sheet acknowledging that they have received lab-specific training and are aware of the safety features in the NPC Laboratories.

DEFINITIONS

Sample: Any biological material received from an outside laboratory or stored material in the NPC.
BSL: Bio-safety-level (see this folder in the handout from the Biosafety ABC for more information)
NPC: Nano and Pico Characterization Lab

REFERENCES

Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, especially section III and appendix H, which are attached to this SOP, available online at http://www.cdc.gov/biosafety/publications/bmbl5/bmbl.pdf and are to be reviewed by any User prior to working in the NPC laboratory.

MATERIALS and/or EQUIPMENT

This SOP applies to ALL equipment in the NPC Lab and ALL experiments.

PROCEDURES

Universal Precautions are suitable for activity involving any BSL-2 grade material. Biosafety Level 2 (BSL 2) represents the laboratory safety level that corresponds to Universal Precautions. Use UNIVERSAL PRECAUTIONS as set forth below and apply to ALL samples:

It is important to understand that at these levels we are dealing with research, diagnostic or production activities thought to pose little or minimal hazards to workers. To date, these procedures and practices have been considered sufficient to protect the work force.

- Persons under the age of 16 are not allowed.
- No food or drinks are allowed (neither consumption nor storage) in the laboratory, neither is the application of make-up, lip balm or smoking.

The control of potential biohazards at the BL-2 level is provided by use of standard microbiological practices with the addition of personnel protective equipment (lab coat and gloves). **Users must bring their own PPE (listed below) and biohazardous waste containers. Users are also responsible for autoclaving and disposing of their own waste.**

- Wear protective body clothing when there is a potential for splashing or spraying samples. Wash hands or other skin surfaces thoroughly and immediately if contaminated with samples.
- **Closed toe shoes** without high heels are required at all times.

- **Clothing that covers the legs fully** is required at all times.
- **Wear face/eye protection** (surgical mask and safety goggles/glasses or a face shield) during procedures that are likely to generate droplets of fluids to prevent exposure to mucous membranes of the mouth, nose, and eyes.
- All persons processing laboratory specimens should wear **gloves**. Gloves should be changed and hands washed if the gloves are crossly contaminated or damaged and after completion of sample processing.
- A special word about gloves: OSHA and CDC propose that gloves be worn when there is potential or hand skin contact with samples, potentially infectious material, or surfaces contaminated with these materials. Gloves should be worn when:

    - Performing routine laboratory work with blood, potentially infectious body fluids, and tissues and all other samples.
    - Handling items (potentially) contaminated with blood or body fluids, including sample container or plates, laboratory instruments, counter tops, etc.
    - Gloves should be changed immediately if contamination is visible.
    - Wash hands immediately after gloves are removed.

- All specimens of cell culture or any other samples should be placed into a well-constructed container or plate with a secure, well-fitting lid.
- Avoid contaminating the outside of the container or plate.
- Take care to minimize the formation of droplets, spatters, splashes. Class 2 Biological Safety Cabinets should be used whenever there is a high potential to produce droplets or aerosols. This includes non-contact dispensing, blending, sonicating, vigorous mixing (vortexing), and homogenization. Biological safety cabinets are not needed for other routine work. Other primary containment devices (e.g., plates with lids or centrifuge safety cups with lid) should be used when manipulating samples outside a biological safety cabinet.
- Mouth pipetting must NOT be done. Mechanical pipetting devices must be for all liquids in the laboratory.
- Laboratory work surfaces should be decontaminated after a spill of any sample and at the completion of work. Decontaminate all instruments and work area after usage with fresh 10% bleach and 70% Ethanol or with CaviCide disinfectant. **External users must bring in their own specific disinfectant for the type of infectious agent that they are working with.** NPC will only provide Bleach, Ethanol, and CaviCide.
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- Contaminated clothing is processed according to institutional policy. See hygiene plan and Blood Born Pathogen control plan for details.
- Laboratory equipment should be cleaned and decontaminated prior to being repaired or transported to the manufacturer.
- The use of any sharps is strongly discouraged at the NPC Lab. In the unusual event that sharps are necessary, take extraordinary care to avoid accidental injuries when performing procedures, cleaning instruments, handling sharp instruments, and during disposal. Place used needles, disposable syringes, scalpel blades, and other sharp items into a puncture-resistant biohazard container or plate (sharps container or plate) for disposal. The container or plate should be located as close as possible to the work area.
- All persons should wash their hands before leaving the laboratory. Protective clothing should be removed before leaving the laboratory.

CONTROLLED OF ACCESS TO NPC LABORATORIES

Access to the NPC Laboratories is controlled by a building wide card reader system. Access cards are provided by the CNSI at the specific request of the NPC management to users to labs which contain instruments or equipment on which they have been trained and when they have completed the NPC lab specific safety training. Cards are set to expire one year from date of issue and must be renewed by specific request to the NPC management.

PRECAUTIONS

For project specific precautions see, read and understand the individual PI's SOP.

EMERGENCIES (SPILL, EXPOSURE and MEDICAL):

Dial 911 (from a campus phone) or 310-825-1491 from a cell phone.

Adam Stieg: 310-206-2902

Shivani Sharma: 310-983-1027

Mike Lozano: 310-345-4835

Emergency contact information posted throughout the laboratory.

GENERAL SPILL PROCEDURE

1. Place paper towel over spill to contain.
2. Spray towel with diluted fresh 30% bleach to disinfect (Contact time at least 10 minutes)
3. Clean up area with water, then 70% Ethanol (Contact time at least 10 minutes)

There are as well spill cleanup procedure posters posted throughout the laboratory in case of emergency.

EXPOSURES

There are multiple eye wash stations and a safety showers located within the main labs. Immediately rinse your body or eyeballs thoroughly for at least 15 minutes. First aid kits are readily accessible for all users. Seek medical attention immediately. For exposures and further details refer to the SOP of the PI from which the sample originated.

MEDICAL

Medical Emergency - Dial 911 or x52111

In case of an acute exposure or medical emergency, Users should seek Emergency Care*** at the following locations:

Life Threatening Emergency, After Hours, Weekends And Holidays – Dial **911** (or 310-825-1491 from cell phone) or contact the Ronald Reagan UCLA Medical Center (emergency room) directly at **x52111** (located at 757 Westwood Plaza, enter from Gayley Avenue). *Note: All serious injuries must be reported to EH&S at **x59797** within 8 hours.*

Non-Life Threatening Emergency – Go to the Occupational Health Facility (OHF), **x56771,** CHS room 67-120 (This is on the 6th floor, 7th corridor, room 120. Enter through the School of Dentistry on Tiverton Drive and proceed to the "C" elevator to the 6th floor.)Hours: M - F, 7:00 a.m.to 4:00 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All injuries must be reported to EH&S at x59797 within 8 hours.*

Needle stick/puncture exposure (as applicable to chemical handling procedure) – Wash the affected area with antiseptic soap and warm water for 15 minutes. For mucous membrane exposure, flush the affected area for 15 minutes using an eyewash station. Hours: M – F, 7:00 a.m. to 4:00 p.m. At all other times report to Ronald Regan UCLA Medical Center (emergency room) at **x52111**. *Note: All needle stick/puncture exposures must be reported to EH&S at x59797 within 8 hours.*

UCLA Users

- Staff, Graduate Students and Undergraduate Students
    - Occupational Health Facility, 67-120 CHS, x56771
    - Mon – Fri 7:30 am – 4:30 pm
- After hours, holidays, weekends, or for major emergencies UCLA Emergency Room in the Ronald Reagan Medical Center

NOTE: Non-UCLA Users are not eligible for emergency medical treatment at the Occupational Health Facility. In the event of an emergency, non-UCLA Users should report to the Ronald Reagan Emergency Room.

REPORTING

Report all spills, exposures or accidents to their Principal Investigator, EH&S Biosafety Office (310-825-5689) and Dr. Adam Stieg immediately (310-206-2144 or 310-206-2902).

WASTE DISPOSAL

All biohazard waste is disposed of using universal precautions as set forth above. Refer as well to the biohazard waste disposal poster posted in the lab. Full wastebags should be tied with a square not, placed in a secondary, leakproof, rigid biohazard container closed with a lid before being removal using a sturdy laboratory cart.

All BSL2 biohazardous samples and plates must be removed from the NPC facility in closed biohazard labeled containers and be disposed of using the biohazard waste containers in the User's own laboratory in accordance with UCLA EH&S policies.

Other contaminated biohazards waste (micropipette tips, etc.) must be disposed of using the biohazard waste containers located in the NPC laboratory. Disposal of biohazard waste in these containers in accordance with UCLA EH&S policies is the responsibility of the NPC laboratory staff.

If the outside of the container is contaminated, it cannot be removed from the laboratory but needs to be decontaminated according to UCLA EH&S practices.

APPENDIX

WORKING WITH HUMAN, NHP or MAMALLIAN CELLS AND TISSUES

Although risk of laboratory infection from working with cell cultures in general is low, risk increases when working with human and other primate cells, and primary cells from other mammalian species. There are reports of infection of laboratory workers handling primary rhesus monkey kidney cells,[1] and the bloodborne pathogen risks from working with primary human cells, tissues and body fluids are widely recognized.[2,3] OSHA has developed a bloodborne pathogens standard that should be applied to all work in the laboratory with human blood, tissues,

body fluids and primary cell lines.[4] Procedures have also been published to reduce contamination of cell cultures with microorganisms.[5,6]

POTENTIAL LABORATORY HAZARDS

Potential laboratory hazards associated with human cells and tissues include the bloodborne pathogens HBV, HIV, HCV, HTLV, EBV, HPV and CMV as well as agents such as *Mycobacterium tuberculosis* that may be present in human lung tissue. Other primate cells and tissues also present risks to laboratory workers.[7] Cells immortalized with viral agents such as SV-40, EBV adenovirus or HPV, as well as cells carrying viral genomic material also present potential hazards to laboratory workers. Tumorigenic human cells also are potential hazards as a result of self-inoculation.[8] There has been one reported case of development of a tumor from an accidental needle-stick.[9] Laboratory workers should never handle autologous cells or tissues.[1] NHP cells, blood, lymphoid and neural tissues should always be considered potentially hazardous.

RECOMMENDED PRACTICES

Each institution should conduct a risk assessment based on the origin of the cells or tissues (species and tissue type), as well as the source (recently isolated or well characterized). Human and other primate cells should be handled using BSL-2 practices and containment. All work should be performed in a BSC, and all material decontaminated by autoclaving or disinfection before discarding.[6,10,11,12] BSL-2 recommendations for personnel protective equipment such as laboratory coats, gloves and eye protection should be rigorously followed.

All Users and laboratory staff working with human cells and tissues should be enrolled in an occupational medicine program specific for bloodborne pathogens and should work under the policies and guidelines established by the institution's Exposure Control Plan.[4] Laboratory staff and Users working with human cells and tissues should provide a baseline serum sample, be offered hepatitis B immunization***, and be evaluated by a health care professional following an exposure incident. Similar programs should be considered for work with NHP blood, body fluids, and other tissues.

*****NOTE: Non-UCLA Users are not eligible for evaluations or vaccinations through UCLA Occupational Health. All pre-entrance medical evaluations will need to be handled through the User's employer.*****

REFERENCES

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10. Barkley WE. Safety considerations in the cell culture laboratory. Methods Enzymol.1979;58:36-43.

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PRINCIPLES OF BIOSAFETY

A fundamental objective of any biosafety program is the containment of potentially harmful biological agents. The term "containment" is used in describing safe methods, facilities and equipment for managing infectious materials in the laboratory environment where they are being handled or maintained. The purpose of containment is to reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potentially hazardous agents. The use of vaccines may provide an increased level of personal protection. The risk assessment of the work to be done with a specific agent will determine the appropriate combination of these elements.

LABORATORY PRACTICE AND TECHNIQUE

The most important element of containment is strict adherence to standard microbiological practices and techniques. Persons working with infectious agents or potentially infected materials must be aware of potential hazards, and must be trained and proficient in the practices and techniques required for handling such material safely. Responsibility for providing, arranging and documenting the appropriate training of Users is the responsibility of their respective PI, not the NPC Laboratory. The NPC Director is responsible for providing or arranging the appropriate training of lab staff/personnel as well as maintaining their training records.

Each laboratory should develop or adopt a biosafety or operations manual that identifies the hazards that will or may be encountered, and that specifies practices and procedures designed to minimize or eliminate exposures to these hazards. Personnel should be advised of special hazards and should be required to read and follow the required practices and procedures. A scientist, trained and knowledgeable in appropriate laboratory techniques, safety procedures, and hazards associated with handling infectious agents must be responsible for the conduct of work with any infectious agents or materials. This individual should consult with biosafety or other health and safety professionals with regard to risk assessment.

When standard laboratory practices are not sufficient to control the hazards associated with a particular agent or laboratory procedure, additional measures may be needed. The laboratory Director is responsible for selecting additional safety practices, which must be in keeping with the hazards associated with the agent or procedure. Laboratory personnel, safety practices, and techniques must be supplemented by appropriate facility design and engineering features, safety equipment, and management practices.

SAFETY EQUIPMENT (PRIMARY BARRIERS AND PERSONAL PROTECTIVE EQUIPMENT)

Safety equipment includes BSCs, enclosed container or plates, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures. Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A. Open-fronted Class I and Class II BSCs are primary barriers that offer significant levels ofprotection to laboratory personnel and to the environment when used with good microbiological techniques. The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological

stocks) being manipulated inside the cabinet. The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

An example of another primary barrier is the safety centrifuge cup, an enclosed container or plate designed to prevent aerosols from being released during centrifugation. To minimize aerosol hazards, containment controls such as BSCs or centrifuge cups must be used when handling infectious agents.

Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Personal protective equipment is often used in combination with BSCs and other devices that contain the agents, animals, or materials being handled. In some situations in which it is impractical to work in BSCs, personal protective equipment may form the primary barrier between personnel and the infectious materials. Examples include certain animal studies, animal necropsy, agent production activities, and activities relating to maintenance, service, or support of the laboratory facility.

FACILITY DESIGN AND CONSTRUCTION (SECONDARY BARRIERS)

The design and construction of the facility contributes to the laboratory workers' protection, provides a barrier to protect persons outside the laboratory, and protects persons or animals in the community from infectious agents that may be accidentally released from the laboratory. The Laboratory Director is responsible for providing facilities commensurate with the laboratory's function and the recommended biosafety level for the agents being manipulated.

The recommended secondary barrier(s) will depend on the risk of transmission of specific agents. For example, the exposure risks for most laboratory work in BSL-1 and BSL-2 facilities will be direct contact with the agents, or inadvertent contact exposures through contaminated work environments. Secondary barriers in these laboratories may include separation of the laboratory work area from public access, availability of a decontamination facility (e.g., autoclave), and hand washing facilities.

When the risk of infection by exposure to an infectious aerosol is present, higher levels of primary containment and multiple secondary barriers may become necessary to prevent infectious agents from escaping into the environment. Such design features include specialized ventilation systems to ensure directional air flow, air treatment systems to decontaminate or remove agents from exhaust air, controlled access zones, airlocks as laboratory entrances, or separate buildings or modules to isolate the laboratory. Design engineers for laboratories may refer to specific ventilation recommendations as found in the *ASHRAE Laboratory Design Guide* published by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE).[1]

BIOSAFETY LEVELS

Four BSLs are described here, which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed, the documented or suspected routes of transmission of the infectious agents, and the laboratory function or activity. The BSLs described in this manual should be differentiated from Risk Groups, as described in the *NIH Guidelines* and the World Health Organization Laboratory Biosafety Manual. Risk groups are the result of a classification of microbiological agents based on their association with, and resulting severity of, disease in humans. The risk group of an agent should be one factor, to be considered in association with mode of transmission, procedural protocols, experience of staff, and other factors in determining the BSL in which the work will be conducted.

The recommended biosafety level(s) for the organisms in Section 8 (Agent Summary Statements) represent those conditions under which the agent ordinarily can be safely handled. Of course, not all of the organisms capable of causing disease are included in Section 8 and an institution must be prepared to perform risk assessments for these agents using the best available information. Detailed information regarding the conduct of biological risk assessments can be found in Section 2. The laboratory Director is specifically and primarily responsible for assessing the risks and applying the appropriate biosafety levels. The institution’s Biological Safety Officer (BSO) and IBC can be of great assistance in performing and reviewing the required risk assessment. At one point in time, under the *NIH Guidelines*, BSOs were required only when large scale research

or production of organisms containing recombinant DNA molecules was performed or when work with recombinant DNA molecules was conducted at BSL-3 or above. IBCs were required only when an institution was performing non-exempt recombinant DNA experiments. Today, however, it is strongly suggested that an institution conducting research or otherwise working with pathogenic agents have a BSO and properly constituted and functioning IBC. The responsibilities of each now extend beyond those described in the *NIH Guidelines* and depend on the size and complexity of the program. Generally, work with known agents should be conducted at the biosafety level recommended in Section 8. When information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified. Often an increased volume or a high concentration of agent may require additional containment practices.

Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis, Naegleria gruberi,* infectious canine hepatitis virus, and exempt organisms under the *NIH Guidelines* are representative of microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple *in vivo* passages should not be considered avirulent simply because they are vaccine strains. BSL-1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level. BSL-2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA *Bloodborne Pathogen Standard* 2 for specific required precautions).

Primary hazards to personnel working with these agents relate to accidental percutaneous or mucous membrane exposures, or ingestion of infectious materials. Extreme caution should be taken with contaminated needles or sharp instruments. Even though organisms routinely manipulated at BSL-2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential that may increase the risk of such personnel exposure must be conducted in primary containment equipment, or in devices such as a BSC or safety centrifuge cups. Personal protective equipment should be used as appropriate, such as splash shields, face protection, gowns, and gloves. Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.