

APPENDIX D: HAZARD CONTROL PLAN FOR BSL-2 FACILITIES AT LANL.

The following Hazard Control Plan, B-HRL-009-00, entitled “Biosafety Level 2 – Cell/Microbe Culture,” is currently under review for renewal. It is expected that hazard control plans will be periodically reviewed and updated as needed; they are, therefore, considered to be “living documents.” The subject hazard control plan expires in February 2002 and will be replaced. This plan is therefore included as an example of such a plan and should not be confused with the actual hazard control plan under which the LANL BLS-2 facilities may be currently operating. Attachments to the subject hazard plan have not been included herein. A hazard control plan would be written and implemented appropriate to BSL-3 facilities should NNSA proceed with implementation of the proposed BSL-3 facility at LANL.

Hazard Control Plan

Bioscience Division

B-HRL-009-00

TITLE OF WORK: BIOSAFETY LEVEL-2 CELL/ MICROBE CULTURE

Activity/ Task Identification Number(s): B-HCP-009-00

Statement of work:

This plan covers the culture/growth of cells that could potentially carry pathogenic disease organisms, the growth of infectious microbes, and recombinant DNA from infectious organisms. Culture and growth of cells and microbes is a standard part of basic biology and biochemistry research. Work with non-infectious organisms and cells (Biosafety Level-1) have been characterized in HCP-001-00. The culture of potentially infectious materials changes the risk to the worker, the public and the environment. Handling of potentially infectious materials requires a higher level of stringency be applied to operating procedures. The Center for Disease Control (CDC) in Atlanta, Georgia in conjunction with the National Institutes of Health (NIH) have developed a system of categorizing microbiological work according to the pathogenicity of the organisms being handled. Standard culture work is conducted at Biosafety Level -1, the least hazardous level (HCP-001-00). Biosafety Level-2 adds more stringent access, posting, procedures and engineering controls to the conduct of work. It also requires control of experimental wastes by decontamination prior to disposal.

Culture of pathogenic microbes (bacteria, fungi or viruses) can be safely conducted once the appropriate biosafety procedures and engineering controls are applied. Data collected over the past twenty years from medical laboratories handling infectious materials has shown these methods protect workers and the public. Engineering controls include restricting access to the area and conducting work in HEPA filtered biohoods. To conduct work at BSL-2, best laboratory practices apply and are coupled with engineering controls, decontamination of wastes and good housekeeping. Prior to the conduct of any cell or microbe culture work, protocols describing specific safeguards must be reviewed and approved by LANL's Institutional Biosafety Committee (IBC). Protocols that remain in use over multiple years must be re-approved annually. The IBC assures the institution, the public and workers that experimental work is done at the appropriate biosafety level.

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Initial Risk Estimate: **MEDIUM**

Hazard Control Plan

Bioscience Division

B-HRL-009-00

List of Work Permits Required to Perform Work:

- IBC APPROVAL
- HUMAN SUBJECTS REVIEW BOARD APPROVAL IF
SUBJECT-SPECIFIC HUMAN CELLS ARE USED
- ANIMAL CARE AND USE COMMITTEE APPROVAL IF REQUIRED

Residual Risk Estimate: **MINIMAL**

THIS WORK REQUIRES DIVISION DIRECTOR AUTHORIZATION.

Authorization Expiration Date: **February 2002**

Hazard Control Plan

Bioscience Division

B-HRL-009-00

1. Work Definition

A. Summary of Statement of Work

Biochemical and microbiological research involving the growth, amplification and characterization of a variety of microorganisms, cellular organelles and biomolecules (proteins, nucleic acids, carbohydrates and lipids) occurs in many labs in HRL-1 and -20. Some labs have both BSL-1 and BSL-2 activities because the handling of potentially infectious materials or recombinant DNA is very limited or periodic. When handling involves potentially infectious materials the labs are posted. Specific tasks for the work vary according to the materials being handled they involve common techniques and operations referred to as "universal precautions" which are sufficient to contain the work. This permits the handling of "unknown" samples, which might or might not be infectious or harbor infective microbes. Work with these materials can be intermittent making lab areas BSL-2 on a temporary basis. Other labs are full-time BSL-2. All labs are posted when BSL-2 work is being conducted. Liquid wastes from BSL-2 operations must be decontaminated with bleach or disinfectant prior to disposal. All solid wastes must be autoclaved (heat sterilized) prior to disposal.

B. Definition of tasks (complete set of techniques- individual tasks vary)

1. Design and plan experiments, submit for IBC review/ approval
2. Inoculation and growth of cell/ microbial cultures using universal procedures
3. Isolation of cells/ microorganisms from growth media
4. Cell/ microbe lysis and sub cellular fractionation (varies according to experimental design). Sub cellular fractions (macromolecules) are NOT INFECTIOUS.
5. Clean-up of work areas to assure decontamination, wastes are deactivated by use of microbiocidal chemicals (bleach) or by heat-sterilization. Work surfaces inside biohoods must be sterilized with chemical cleaners or 70% alcohol then irradiated with ultra-violet light.
6. Personal hygiene (hand washing, lab coat) to assure no transfer of organisms to worker or personal clothing
7. Wastes must be decontaminated prior to disposal, add bleach/disinfectant to liquids and autoclave solids.

Tasks can take from minutes to hours. Growth of cultures can take hours to months. Tasks are routinely part of a cascade. Success or completion of experimental plans is dependent on completion of each individual task, resulting in considerable time and materials investment in each successive task. Careful work is required in all tasks.

Hazard Control Plan

Bioscience Division

B-HRL-009-00

C. Instrumentation/ equipment used to execute work (not all are required)

1. Personnel Protective Equipment (eye protection, gloves, close-toed shoes, and protective clothing/ lab coat) where required
2. Protective barriers (biohoods)
3. Incubators and fermentors
4. Heating baths and blocks from ~40 degrees centigrade (C) - 100 degrees C
5. Centrifuges - low-medium- and ultra-speeds
6. Cell disruption devices (sonication or eletroporation)
7. Chromatography instruments
8. Spectrometers
9. Inert cryogenes (liquid nitrogen, dry ice)
10. Compressed gas cylinders
11. Automated Sequencers
12. Electrophoresis instruments
13. Microscopes (light and fluorescence)
14. Ultra-violet light
15. Microwave ovens (heating medias, buffers and gels)
16. Vacuum pumps
17. Automated X-ray film processor or manual development process
18. pH meters
19. Balances
20. Open flames
21. Cold Room Work
22. Cell Counters
23. Pipettors
24. Vortex mixers

E. Location of Work

TA-43-HRL-1 260, 243, 127, 131, 126, B244, B116
TA-43-HRL-20 B104

F. Personnel Performing Work

Students, Technicians, Post-Doctoral appointees, Technical Staff Members and visitors who are trained to do this work either by completion of college level course work, degree and/or task-specific training. ALL PERSONNEL MUST BE IDENTIFIED IN IBC PROTOCOLS.

G. Constraints of the Facility and/or Location

1. Access to laboratory areas of HRL-1 and -20 is restricted to badge- or key-holders only. Access may be affected by badge-reader failure. All doors function with keys.

Hazard Control Plan

Bioscience Division

B-HRL-009-00

2. All biohoods hoods must be performance certified annually . Biohoods are unfunctional if electrical service fails.
3. Sinks drain to the sanitary sewer, no chemical disposal is permitted. Disposal into the sinks of growth medias, that do not contain hazardous materials and have disinfectant (10% bleach/ commercial disinfectant) added, is permitted.
4. Personnel comfort is maintained between 68 and 80 degrees, with about 6 changes of air per hour. Extremes of either cold or heat may occur during mechanical failures and will NOT require shutdown of work. Personnel may have to accommodate discomforts and scale work accordingly.
5. Exhaust from rooms stops if electrical service or mechanical systems fail.
6. All instrumentation, including autoclaves, utilize electrical service and will be inoperable if electrical service fails.
7. In the event of fire alarm or power failure during handling operations, cultures must be decontaminated immediately with addition of bleach or disinfectant.
8. Freezers containing valuable research materials can fail if electrical service is lost. Some units have "back-up power " to assure materials are not lost.

H. Effects on the Environment - None expected.

I. Legacy Issues Associated with Work - None expected

2. IDENTIFICATION OF HAZARDS AND CONTROLS

BIOLOGICAL HAZARDS

All work with biological materials must comply with LANL's Biosafety LIR (402-530-00). Work with primary cells is always assumed to have the potential for the presence of infectious organisms and is conducted at BSL-2 at a minimum. "Universal precautions" are required as defined in "Biosafety in Microbiology and Medical Laboratories" (ATTACHMENT A). Work with suspect or known pathogenic organisms at laboratory scale (in small volumes, not production) also requires this level of safety. Work with recombinant DNA from infectious organisms might require BSL-2. Work with biotoxins IS COVERED IN A SEPARATE HCP. All work has an Exposure Control Plan (ECP, ATTACHMENT B).

Hazard Control Plan

Bioscience Division

B-HRL-009-00

PHYSICAL HAZARDS

- | | | |
|---|--|--|
| <input checked="" type="checkbox"/> Compressed Gases | <input checked="" type="checkbox"/> Cryogenics | <input checked="" type="checkbox"/> Hot Surfaces |
| <input checked="" type="checkbox"/> Ergonomics | <input type="checkbox"/> Lifting/Carrying | <input type="checkbox"/> Noise |
| <input type="checkbox"/> Projectiles | <input checked="" type="checkbox"/> Sharps | <input checked="" type="checkbox"/> Open Flames |
| <input checked="" type="checkbox"/> Pressurized Systems | <input checked="" type="checkbox"/> Rotating/Reciprocating Systems | |
| <input type="checkbox"/> Temperature Extremes | <input type="checkbox"/> Working at Heights | <input type="checkbox"/> Confined Space |

TYPE	CONTROLS
Compressed Gases on can poise several safety concerns.	Use with appropriate regulator, transport on cart with cap and properly secured. Reference "Gas Cylinder Handling" procedure B-.003-99
Cryogenics can burn skin or eyes and can cause frostbite.	Use thermal gloves and face shield, reference procedure B-0004-99.
Hot Surfaces can burn skin or ignite fires.	Reference "Heating Procedures" B-007-99
Ergonomic injuries cause long-term soft tissue injury.	Long-duration repetitive tasks should be avoided and can workstations should promote good posture.

TYPE	CONTROLS
Sharps can cut or puncture skin.	Handle all sharps carefully, reference "Sharps Handling" procedure, LS-008-99
Open Flames can burn or ignite fires.	Reference LS SOP for "Open Flames".
Pressurized Systems can explode.	Autoclaves are high pressure, high temperature systems. Reference LS Autoclave HCP

Hazard Control Plan

Bioscience Division

B-HRL-009-00

Rotating/ Reciprocating equipment can release projectiles or entangle fingers and arms. Typically these are centrifuges. Follow manufacturers' instructions keep units clean and well serviced.

RADIATION HAZARDS

Criticality Ionizing Radiation Lasers
 High Intensity Light Radio-frequency/Microwave
 Ultra-violet Light Infrared Radiation

<u>TYPE</u>	<u>CONTROLS</u>
Microwave ovens can overheat liquids causing containers to break or explode.	Reference "Heating Operations" LS-007-99.
Ultra-violet Light can burn eye and skin.	Shield eyes and skin, wear safety glasses and lab coats.

ELECTRICAL HAZARDS (Definitions in Electrical Safety LIR)

Electromagnetic Energized Equipment Shock

<u>TYPE</u>	<u>CONTROLS</u>
Shock can be debilitating opportunities even lethal. manufacturers' for condition.	Instrumentation and electrical service provides for contact, with electricity follow instructions and check cords

Hazard Control Plan

Bioscience Division

B-HRL-009-00

CHEMICAL HAZARDS

<input type="checkbox"/> Asphyxiant	<input type="checkbox"/> Beryllium	<input checked="" type="checkbox"/> Carcinogen
<input checked="" type="checkbox"/> Reproductive Toxin	<input checked="" type="checkbox"/> Highly Toxic	<input checked="" type="checkbox"/> Dusts/Powders
<input type="checkbox"/> Corrosive	<input type="checkbox"/> Hazardous Gas	<input checked="" type="checkbox"/> Ignitable
<input checked="" type="checkbox"/> Incompatible	<input checked="" type="checkbox"/> Irritant	<input checked="" type="checkbox"/> Mutagen
<input type="checkbox"/> Poison	<input type="checkbox"/> Oxidizer	<input type="checkbox"/> Other

TYPE	CONTROLS
Carcinogens can have effects on cells that lead to cancer.	Use is with micro-quantities, check MSDS, SOP(s) may apply.
Reproductive Toxins can affect fertility.	Use is with micro-quantities, check MSDS, SOP(s) may apply.
Highly Toxic materials poisons with immediate or near-term health effects.	Use is with micro-quantities, check MSDS, SOP(s) may are apply.
Dusts/ Powders can be allergenic or irritants.	Reference MSDS.
Ignitable materials can cause burns or fires.	Reference MSDS and "Open Flame Operations" Bioscience procedures,
Incompatibles can explode or have other violent reactions.	Reference MSDS
Irritants can cause skin, eye or mucous membrane damage.	Reference MSDS
Mutagens can cause cell damage with long-term potential for health effects.	Reference MSDS

Hazard Control Plan

Bioscience Division

B-HRL-009-00

ENVIRONMENTAL HAZARDS:

Asbestos Infectious Waste Air Emission
 Hazardous Waste Waste water
 Polychlorinated Biphenyls

<u>TYPE</u>	<u>CONTROLS</u>
Hazardous Wastes	Chemical wastes only. Biological materials in BSL-2 rooms wastes are autoclaved or treated with bleach. NO INFECTIOUS WASTES ARE GENERATED , decontaminated wastes (solid/liquid) are disposed of as non-hazardous biological materials.

TRANSPORTATION

Hazardous Materials Radiological Materials

Samples of experimental materials, typically DNA and mammalian cells, are shipped to and received from collaborators worldwide. All transport is according to DOT REGULATIONS AND LANL LIR 405-10-01. Some samples are shipped on dry ice, always less than 2 pounds. Samples being transported by researchers off-site conform to LIR 405-10-01. Samples are sent to HRL from collaborators and others, they conform to DOT regulations as managed by their institutions at point of origin.

3. RISK ASSESSMENT - By "What-If Checklist" Method

A. DETERMINATION OF *INITIAL RISK*: **MEDIUM**

The handling of primary human cells, cells from primary tissues of animals or humans and the culture of potentially infectious microorganisms can result in workers or the public contracting diseases. Therefore, the culture of potentially infected cells or known pathogenic microbes presents opportunities for transmission of infection from the work materials to the worker or their families or others. This level of safety permits handling of unknowns-, which could be harboring lethal organisms. The likelihood of accidental exposure would be occasional, but the severity would be critical.

Hazard Control Plan

Bioscience Division

B-HRL-009-00

B. DETERMINATION OF *RESIDUAL RISK*: MINIMAL

Medical laboratory experience has demonstrated that Biosafety Level-2 containment practices dramatically diminish the occurrence of worker exposure or infection. Using the techniques provided by CDC/NIH in their publication, "Biosafety in Microbiology And Medical Laboratories", risk of exposure or infection is minimal. Vaccinations can be provided to further protect workers from unnecessary risks. The likelihood of getting exposed to sufficient quantities of infectious organisms is reduced to remote, but the consequences of any such exposure remains critical, particularly for lethal organisms.

-8- HAZARD CONTROL PLAN

4.0 Exposure Control Plan (ECP) for Bioscience workers who conduct work with human blood, tissues or fluids.

A. Roles and Responsibilities:

1) Institutional

- LANL has chartered an IBC, which must review all work for safety concerns and approve work as described.
- LANL provides an Occupational Medicine program, ESH-2, to support research programs where potential exposure to blood-borne pathogens exists.

2) Organizational

- Bioscience managers will direct workers to an occupational medicine program that, at a minimum, includes medical evaluations prior to, during and post- work.
- Bioscience managers will provide a safe environment for the work including administrative and engineering controls as appropriate, and suitable PPE.
- Bioscience managers will provide explanations of work including information on specific pathogens being handled or having the potential to be handled.

Hazard Control Plan

Bioscience Division

B-HRL-009-00

- Bioscience managers will provide, through ESH-2, free vaccination programs for affected workers who want to participate.
- Bioscience managers will provide for safe waste handling.
- Bioscience managers will provide training programs and training opportunities.
- Bioscience managers will post all work areas appropriately.

3) Workers

- will conduct work according to HCPs and/ or applicable protocols using "Universal Precautions"
- will handle wastes as required
- will use appropriate PPE
- will use engineering controls provided
- will adhere to postings
- will maintain good housekeeping in work areas
- will wash hands after work

B. Training and Information

1) ATTACHMENT C is 29 CFR 1910.1030

2) Written or oral work plans will include:

- explanation of symptoms and epidemiology of blood borne diseases
- modes of transmission
- explanations of tasks and modes of potential exposures
- explanation of use and limitations of controls
- complete review of the location, handling, decontamination and disposal of PPE
- explanation of the basis for selection of PPE
- information on available vaccinations including efficacy, safety, and benefits
- review of appropriate emergency procedures

-9

HAZARD CONTROL PLAN

C. Emergency Procedures:

1) In case of facility failure (loss of electricity for lighting and equipment) work must stop, materials must be secured either by closures of containers or addition of disinfectant then surfaces must be decontaminated. Access must be restricted and postings remain until normal operations resume. If evacuation occurs, provide specific information about materials being used and status of the space to emergency responders.

2) In case of fire concerns for personnel safety must prevail. Evacuate promptly if possible add disinfectant to any open containers. Should a fire begin at the site of this work workers may choose to attempt containment of the flames or evacuation and should not place themselves or others in danger. Evacuate and

Hazard Control Plan

Bioscience Division

B-HRL-009-00

find closest fire pull box, turn in alarm. Provide information about the specific materials in use and the status of the space for emergency responders.

3) In case of accidental spill or loss of containment add disinfectant (bleach or specific commercial product) to area. Close all open containers. Contain spill with barriers. Get help from co-workers to assure access control and notification of line managers and facility manager, but avoid opportunities for personnel exposures. Remove PPE, wash hands report incident to line manager and/ or facility manager. Notify EM&R (667-6211 or 911) if volumes or conditions of spill prevent local containment.

4) In case of exposure to workers remove contaminated clothing, wash exposed skin and report to Occupational Medicine (ESH-2) immediately, or go directly to Los Alamos Medical Center. See that your supervisor, line manager and facility manager are promptly notified. Other unaffected workers should call 911 to get containment assistance/ guidance. Control access and limit opportunities for others to be exposed.

All incidents that involve loss of containment or exposure MUST be reported to appropriate line managers and the facility manager. Potential loss of containment or exposure should be reported to line manager(s) for the purpose of conducting a lessons-learned evaluation for improvements.

