APPENDIX A
Strategic Plan for Biodefense Research

This document is available online at
http://www.niaid.nih.gov/biodefense/research/strategic.pdf

APPENDIX B
Characteristics of Diseases Studied at RML

Table B-1
Characteristics of Primary Diseases That Are Being Or Have Been Studied at the RML

Disease	Infectious Agent	Occurrence	Reservoir[1]	Transmission[2]	Incubation Period[3]	Communicable Period[4]
Bacterial diseases						
Lyme Disease	*Borrelia burgdorferi*	Along the Atlantic coast, concentrated between Massachusetts and Maryland; upper Midwest; and local areas of California and Oregon. Cases reported from 47 states, Canada. Also occurs in Europe and Asia.	Primarily wild rodents.	Primarily ticks of the genus *Ixodes*.	3-32 days, mean of 7-10 days.	No evidence of person-to-person transmission.
Endemic Relapsing Fever	*Borrelia hermsii*	Endemic in the United States	Rodents and soft-bodied ticks	Ticks *Onitodoros hermsii*	5-15 days.	No person-to-person transmission
Plague	*Yersinia pestis,*	Wild rodent plague occurs in the western U.S.; large areas of South America; north central, eastern, and southern Africa; central and southeast Asia, and south-eastern Europe near the Caspian Sea; and localized areas in the Russian Federation and Kazakhstan. Recent outbreaks of have occurred in in Africa and Asia, and local outbreaks South America.	Wild rodents, rabbits and hares, wild carnivores and domestic cats.	People generally become infected by being bitten by an infected rodent flea or handling an infected animal; rarely by airborne droplets from human patients or household cats with plague pharyngitis or pneumonia.	1-7 days.	Fleas may remain infective for months. Pneumonic plague may be highly communicable under some conditions. Bubonic (swollen lymph nodes) form is rarely transmitted directly.
Tuberculosis (TB)	*Mycobacterium tuberculosis* complex. Includes *M. tuberculosis* and *M. africanum* from humans, *M. bovis* from cattle	Worldwide.	Humans, rarely primates. Possibly diseased cattle, swine, badgers, and other mammals	Coughing or sneezing by people with tuberculosis of the lungs or throat. Rarely transmitted through direct contact with broken skin or mucous membrane. Bovine tuberculosis may be acquired from tuberculosis cattle or unpasturized milk products.	2-10 weeks. Latent (inactive, asymptomatic) infection may persist for a lifetime.	As long as viable tubercle bacilli are being discharged while coughing.

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Disease	Infectious Agent	Occurrence	Reservoir[1]	Transmission[2]	Incubation Period[3]	Communicable Period[4]
Salmonellosis	*Salmonella enterica serovar Typhimurium*	Worldwide	Wide range of domestic and wild animals, including poultry, swine, cattle, rodents, and pets; also infected humans.	Eating contaminated food (raw or undercooked). Fecal-oral transmission from person to person.	6-72 hours	Extremely variable, throughout the course of infection; usually several days to weeks.
Antibiotic-resistant Staphylococcus infection	*Staphylococcus aureus*	Worldwide	Humans, rarely animals	Person-to-person.	Variable and indefinite. Often 4-10 days.	Variable: as long as purulent lesions continue to drain or the carrier state persists.
Streptococcal epidemics and vaccine development	*Streptococcus pyogenes*	Worldwide	Humans	Person-to-person, often through exposure to large respiratory droplets from an infected patient or carrier, or direct contact.	Short; usually 1-3 days.	10-21 days in untreated and uncomplicated cases. Weeks to months in untreated conditions with purulent discharges.
Psittacosis (Parrot fever)	*Chlamydia psittaci*	Worldwide	Primarily parakeets, parrots and love birds; less often in poultry, pigeons, canaries and sea birds.	Inhaling the agent from desiccated droppings, secretions, and dust from feathers of infected birds.	1-4 weeks.	No person-to-person transmission. Infected birds may shed the agent intermittently, and sometimes continuously for weeks to months.
Chlamydial Pneumonia	*Chlamyid pneumoniae,* strain TWAR	Worldwide	Humans; no avian associations, not dogs or cats.	Unknown, possibly direct contact with secretions, spread via particles to which bacteria adhere, and airborne spread.	Unknown, possibly at least 20 days	Unknown, but believed to be 8 months or more.

Table B-1
Characteristics of Primary Diseases That Are Being Or Have Been Studied at the RML

Disease	Infectious Agent	Occurrence	Reservoir[1]	Transmission[2]	Incubation Period[3]	Communicable Period[4]
Conjunctivitis ("pinkeye")	*Chlamydia trachomatis*	Worldwide	Humans	Direct contact with infectious eye or nasal discharges, or contact with contaminated towels or clothing.	5-12 days	As long as active lesions are present.
Sexually transmitted Chlamydia	*Chlamydia trachomatis*	Worldwide	Humans	Person-to-person transmission through sexual intercourse.	7-14 days.	Unknown
Rickettsial diseases						
Q Fever	*Coxiella burnetii*	Reported from all continents. Endemic in areas where reservoir animals are present. Veterinarians, ranchers, farmers, meatpackers, lab workers are at high risk.	Sheep, cattle, goats, cats, dogs, some wild mammals, birds, ticks are natural reservoirs	Commonly transmitted by airborne coxiellae in dust particles contaminated with birth fluids or excreta from infected animals.	usually 2-3 weeks.	Direct person-to-person transmission is unlikely. Possibly through contaminated clothing.
Rocky Mountain Spotted Fever	*Rickettsia rickettsii*	Throughout the U.S, and in Canada, Central and South America.	Ticks, small and medium-sized mammals.	Ticks	3-14 days	No person-to-person transmission.

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Disease	Infectious Agent	Occurrence	Reservoir[1]	Transmission[2]	Incubation Period[3]	Communicable Period[4]
Viral Diseases						
Acquired Immuno-deficiency Syndrome (AIDS)	Human immunodeficiency virus (HIV), a retrovirus. Two serologic types: HIV-1 and HIV-2.	Worldwide	Humans	Person-to-person transmission through sexual contact, sharing HIV contaminated needles and syringes, transfusion of infected blood or its components, transplant of infected tissues or organs. Transmission through bodily secretions has not been reported.	Generally 1-3 months. Time from infection to diagnosis can be <1 year to 15 years or more.	Unknown; presumed to be throughout life.
Non-HIV retroviral infections e.g., (Adult T-cell leukemia, T-cell lymphosarcoma, peripheral T-cell lymphoma)	Retroviruses; e.g. human T-cell lymphotrophic virus (HTLV-I, HTLV-II)	Japan, Caribbean, Pacicfic coast of South America, equatorial Africa, southern USA.	Humans	Infection early in life primarily through breast milk. Also through transfer of blood or blood products, IV drug use, or sexual activity.	Exposure through breast milk leads to tumor development in the adult with a peak at age 50.	Throughout infection.
Aleutian mink disease parvovirus	Parvoviruses	Worldwide	Wild and domestic mink and mustelids	Contact with infected animals through biting, urine and respiratory secretions,	Variable; 20-90 days.	Throughout infection
Rabies	Rabies virus; a rhabdovirus of the genus *Lyssavirus*	Worldwide	Wild and domestic canids, skunks, raccoons, mongooses, and certain bats are primary reservoirs.	Saliva of a rabid animal is introduced by a bite or scratch, rarely through a break in the skin or intact mucous membrane.		While theoretically possible, person-to-person transmission has never been documented.

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Characteristics of Primary Diseases That Are Being Or Have Been Studied at the RML

Disease	Infectious Agent	Occurrence	Reservoir[1]	Transmission[2]	Incubation Period[3]	Communicable Period[4]
Agents of transmissible spongiform encephalopathies (prion diseases). A group of degenerative diseases of the brain associated with an abnormal from of prion protein						
Transmissible Spongiform Encephalopathy (TSE)	Believed to be a self-replicating prion protein.	Reported from countries all over the world. Examples of TSEs include: scrapie, in goats and sheep; bovine spongiform encephalopathy (BSE), in cattle; chronic wasting disease (CWD) of deer and elk; Creutzfeldt-Jakob disease (CJD), variant CJD, Gerstmann-Sträussler-Scheinker syndrome, kuru and fatal familial insomnia in humans.	For human-related TSEs, human cases are the only known reservoir. While there is no documentation of human infection acquired from animals, this has been suggested.	Unknown; but there have been cases of CJD acquired from tissue transplants and grafts to eye and nervous system, injections of growth and other hormones. Variant CJD may be acquired through consumption beef with BSE	15 months to 30 years or more.	Tissues of the central nervous system are infectious throughout illness. Lymph tissues and other organs may be infectious before signs of illness appear.
Scrapie[7], TSE of sheep and goats. Not know to infect humans.	Believed to be a self-replicating prion protein.	Worldwide	Sheep and goats	Through exposure to fluid and tissue of infected sheep or goats.	2-5 years.	Immediately post-partum.

[1] Reservoir of infection – Any animal, plant, plant, soil, or substance (or combination) in which the infectious agent normally lives and multiplies; and serves as a source of infection.

[2] Transmission – Mechanism by which an infectious agent is spread from source or reservoir to another person.

[3] Incubation Period – The time interval between infection and the appearance of the first sign or symptom of the disease.

[4] Communicable Period – The time during which and infections agent may be transferred directly from an infected person to another uninfected person.

Source: APHA. 2000. The control of communicable diseases manual (17th edition), J. Chin, editor. American Public Health Association, 800 I Street, NW, Washington, DC 20001-3710.

Table B-2

Characteristics of Viral Diseases Assigned to Biosafety Level 4.[1]

Disease	Infectious Agent	Occurrence	Reservoir[2]	Transmission[3]	Incubation Period[4]	Communicable Period[5]
Tick-borne encephalitdes a. Central European tick-borne encephalitis (CEE Subtype) b. Russian Spring-Summer Encephalitis (FE Subtype	A complex within the flaviviruses; minor antigenic differences exist. Viruses causing these diseases are closely related.	CEE Subtype Predominates in Europe, while FE Subtype has been found predominantly in the far eastern region of the former Soviet Union.	Ticks or ticks and mammals in combination. Rodents and other small mammals and birds serve as sources of tick infections with CEE and FEE Subtypes.	Bite of an infected tick or by consumption of milk from certain infected animals.	7-14 days.	No direct person-to-person transmission.
Congo-Crimean hemorrhagic fever	Congo-Crimean hemmorhagic fever virus (Bunyaviridae, *Nairovirus*)	Observed in the steppe regions of western Crimea, Kersch Peninsula, Kazakhstan, Uzbekistan, Rostov and Astrakhan regions of Russia, Albania and Bosnia-Herzogovina, Bulgaria, Iraq, Arabian Peninsula, Pakistan, western China, tropical Africa and South Africa.	Hares, birds and *Hyalomma* ticks. Domestic animals may serve as hosts. Hosts are unknown in tropical Africa.	Bite of an infected adult tick. Direct person-to-person transmission through contact with blood and secretions from infected patients. Infection also associated with butchering infected animals.	1-12 days, usually 1-3 days.	During period of infection. Highly infectious in hospital setting; infections are common following exposure to blood and secretions.
Ebola hemorrhagic fever	Ebola virus; a filovirus, related to but antigenically distinct from Marburg virus.	Confirmed cases reported from Africa in the Democratic Republic of the Congo, Republic of the Congo, Gabon, Sudan, Ivory Coast, and Uganda.	Unknown despite extensive studies. Believed to be animal-borne	Person-to-person transmission through direct contact with infected blood secretions, organs or semen. Risk is highest during late stages of illness. Under natural conditions, airborne transmission among humans has not been documented.	2-21 days	As long as blood and secretions contain virus.

Table B-2

Characteristics of Viral Diseases Assigned to Biosafety Level 4.[1]

Disease	Infectious Agent	Occurrence	Reservoir[2]	Transmission[3]	Incubation Period[4]	Communicable Period[5]
Nipah virus encephalitis[7].	Nipah virus; a paramyxovirus	Malaysia	Maybe fruit bats. Infected pigs may serve as a source of human exposure.	Believed to be by transmitted via aerosols, but transmission efficiency from pigs to humans is low. No documented person-to-person transmission.	Unknown	Unknown
South American arenaviral hemorrhagic fevers: a. Argentinian b. Bolivian c. Venezuelan d. Brazilian	Tacaribe complex of arenavirus: a. Junín virus. b. Machupo virus. c. Guanarito virus. d. Sabiá virus.	a. Argentinian pampas. b. Rural northeastern Bolivian c. Venezuelan d. Brazilian	Wild rodents; but unknown for Sabiá virus.	Transmission to humans occurs primarily by inhalation of small particle aerosols derived directly from rodent excreta containing virus, saliva, to body fluids. Virus deposited in the environment may also be infective when ingested or by contact with cuts or abrasions. While uncommon, person-to-person transmission of Machupo virus has been documented in health care and family settings.		
Kyasanur Forest disease	*Flavivirus belonging to the tickborne encephalitis-louping Ill complex.*	Kyasanur Forest of the Shimonga and Kanara districts of Karnataka, India.	Probably rodents, shrews, monkeys, and ticks.	By bite of infective (especially nymphal) ticks; most likely *Haemaphysalis spinigera*.	3-8 days.	Not directly transmitted from person to person. Infected ticks remains so for life.
Omsk hemorrhagic fever	*Flavivirus belonging to the tickborne encephalitis-louping Ill complex.*	Forest steppe regions of western Siberia; within the Omsk, Novosibirsk, Kurgan and Tjumen regions.	Rodents, including muskrat, and ticks.	By bite of infective (especially nymphal) ticks; most likely *Dermacentor reticulates and D. marginatus*. Direct transmission from muskrat to human occurs, with disease in families of muskrat trappers.	3-8 days.	Not directly transmitted from person to person. Infected ticks remains so for life.

Table B-2

Characteristics of Viral Diseases Assigned to Biosafety Level 4.[1]

Disease	Infectious Agent	Occurrence	Reservoir[2]	Transmission[3]	Incubation Period[4]	Communicable Period[5]
Lassa fever	Lassa virus; an arenavirus, serologically related to lymphotocytic choriomeningitis, Machupo, Junín, Guanarito and Sabiá viruses.	Sierra Leone, Liberia, Guinea and regions of Nigeria.	Wild rodents; in west Africa, the *Mastomys* species complex.	Primarily through aerosol or direct contact with excreta of infected rodents deposited on surfaces such as floors and beds or in food and water. Direct contact with blood through inoculation with contaminated needles and pharyngeal secretions or urine of infected patient. Infections can also spread by sexual contact.	6-21 days.	During acute febrile phase when virus is present in the throat. Virus may be excreted in urine of patients for 3-9 weeks from onset of illness.
Marburg fever	Marburg virus; a filovirus, related to but antigenically distinct from Ebola virus.	Zimbabwe, Kenya, Democratic Republic of the Congo. Six cases in Germany and Yugoslavia in 1967 followed exposure to African green monkeys from Uganda.	Unknown despite extensive studies. Believed to be animal-borne	Person-to-person transmission through direct contact with infected blood, secretions, organs or semen. Risk is highest during late stages of illness. Under natural conditions, airborne transmission among humans has not been documented	3-9 days	As long as blood and secretions contain virus

APPENDIX C
Transportation of Agents

TRANSPORTATION OF BIOLOGICAL AGENTS

Biological agents and infectious substances are closely related terms found in transfer and transportation regulations. Biological agents including infectious substances may exist as purified and concentrated cultures but may also be present in a variety of materials such as body fluids, tissues, and soil samples (USDHHS 1999). Federal and state agencies' recognition of these materials as hazardous, results in strict enforcement of regulations applicable to transportation and transfer.

Federal agencies with regulatory authority include the U.S. Department of Transportation (DOT), U.S. Postal Service (USPS), U.S. Department of Health and Human Services (USDHHS), and the International Civil Aviation Organization (ICAO). The National Institutes of Health (NIH) policy manual refers the user to policies specific to transportation and transfer of biological agents that have been developed, and reference requirements of the aforementioned agencies (USDHHS 1998).

Transportation

Transportation refers to packaging and shipping biological agents and materials by land, air, or sea, generally or by a commercial carrier. Regulations concerning transportation of biological agents are aimed at ensuring that the public and workers in the transportation chain are protected from exposure to any agent in the package. Protection during transportation is achieved through:

- Requirements for rigorous packaging that will withstand handling and contain all liquid material within the package without external leakage, and specific requirements of shipping carriers;
- Appropriate labeling with biohazard symbol and other labels to alert workers in the transportation chain to hazardous contents of the package;
- Documentation of hazardous contents of package should such information be necessary in an emergency situation; and,
- Training staff in the transportation chain to respond to emergency situations.

Packaging and Shipping

The safe transportation of hazardous materials is a matter of concern to the public, Congress and Federal, state and local officials. To ensure public safety and minimize risks posed by hazardous materials in transportation, Congress requires the Secretary of Transportation to prescribe regulations for safe transportation of hazardous materials.

The Research and Special Programs Administration is the agency within the Department of Transportation responsible for developing and issuing hazardous materials regulations (HMR: 49CFR Parts 171-180). The HMR govern the classification, hazard communication, and packaging of hazardous materials for transportation.

Also regulating the packaging and shipping of dangerous goods is the International Civil Aviation Organization. The International Air Transport Association (IATA) annually publishes the *IATA Dangerous Goods Regulations.* This manual is based on the International Civil Aviation Organization Technical Instructions. It incorporates additional operational requirements, which provide a harmonized system for operators to accept and transport dangerous goods safely and efficiently. In recent years, the DOT regulations and the ICAO regulations have been revised to reflect, generally, the same requirements.

Infectious substances are one class (Division 6.2) of hazardous materials regulated under these rules. An infectious substance may not be offered for transportation or transported interstate of foreign commerce by rail, water, air or highway unless published requirements are met.

Packaging is the essential component in the safe transport of dangerous goods. The IATA and DOT Regulations provide specific packing instructions for all dangerous goods. The packing instructions require the use of UN performance-tested specification packaging. Infectious substances require tertiary containment where the primary and secondary inner containers are watertight. An inner container must be capable of withstanding internal pressure of 95 kpa at -40 degrees F to 131 degrees F. Outer packaging must be capable of passing a 9 m drop test, penetration testing and a vibration standard. Dangerous goods must be properly identified, classified, packed, marked, labeled, documented and in the condition for transport in accordance with these regulations.

Training is also an essential element in maintaining safe transportation of dangerous goods. It is required that all individuals involved in the preparation or transport of dangerous goods be properly trained to carry out their responsibilities. Depending on the job-function, this may entail only familiarization training or may also include more detailed training in the intricacies of the regulations. It is important to remember that dangerous goods are very unlikely to cause a problem when they are prepared and handled in compliance with DOT and IATA regulations.

The proper declaration of the dangerous goods by the shipper ensures that all in the transportation chain know what dangerous goods they are transporting, how to properly load and handle them and what to do if an incident or accident occurs during transport.

In addition to the IATA and DOT regulations for transport, several agencies of the US Government require permits or declarations for transport or transfer of infectious or hazardous materials:

US Department of Health and Human Services, Public Health Service

US Department of Agriculture, Animal Plant Health Inspection Service, National Center for Import and Export

US Department of Interior, US Fish and Wildlife Service

US Department of Commerce, Export Administration Regulations

Labeling

In accordance with U.S. DOT, Research and Special Programs Administration requirements, as specified under 49 CFR Part 172.323 and 172.432, substances classified as a Biohazard (primarily medical wastes) or as an Infectious Substance (bloodborne pathogens) are specifically labeled. These labels, illustrated in Figure C-1, must be present on two opposing sides or ends of the package, measure a minimum of 6 inches on each side, and must be visible from the direction it faces.

In addition, NIH specifically requires that the correct UN number [i.e. UN2814 (human) or UN2900(animal)] must be recorded on the front of the outside packaging followed by the proper shipping code. All packages must also be marked on the outside with the name and telephone number of a person responsible for the shipment.

Documentation

Several agencies require NIH to obtain specific permits prior to shipment of hazardous biological substances depending on the nature of those substances. Permitting authorities include the following:

- Director, Centers for Disease Control, Public Health Service, USDHHS - pursuant to Section 215 of the Public Health Service Act, as amended (42 U.S.C. 215) and Sections 71.54 and 72.3 of Title 42 of the Code of Federal Regulations (CFR).
- U.S. Department of Agriculture (USDA) regulations (Section 122.2 of Title 9, CFR, and Title 7 CFR Part 330).
- U.S. Fish and Wildlife Service (USFWS), U.S. Department Interior (50 CFR Parts 13 and 14).
- Department of Commerce – the Export Control Act of 1949 (as amended).

- CDC/NIH publication, Biosafety in Microbiological and Biomedical Laboratories provides guidance for classification and containment standards for Biosafety Level (BL) agents 1-4.

Training

Selected research laboratory employees do receive training that is specific to the transportation of bloodborne/airborne pathogens. All research laboratory employees receive overall training and numerous safeguards have been implemented as policy or by law that apply to the handling practices required in the transportation of these materials. Notable regulations administered by the Occupational Health and Safety Administration (OSHA) under 29 CFR Part 1910.1030 include the following:

- Exposure Control Plan, 29 CFR 1910.1030(c)(1): Each employer having an employee(s) with potential for occupational exposure to bloodborne pathogens is required to establish a written Exposure Control Plan designed to eliminate or minimize employee exposure potential. Elements of the Exposure Control Plan include: evaluation of exposure incidents; accessibility to employees; annual review and updates; incorporation of improvements in technology; and, input solicited from non-managerial employees.
- Exposure Determination, 29 CFR 1910.1030(c)(2): Exposure determinations shall be prepared for employees with potential for occupational exposure including lists of job classifications, and specific tasks and procedures that pose potential risk.
- Engineering and Work Practice Controls, 29 CFR 1910.1030(d)(2): Work practice controls shall be used to eliminate or minimize employee exposure. Employees are required to provide facilities and training of employees regarding the following work practices: washing; contaminated sharps disposal; puncture resistant and leakproof containers; prohibited practices in designated areas (e.g., eating, smoking, applying cosmetics); food and drink storage; handling practices to prevent splashing or spattering; container labeling; and, equipment and shipping container decontamination.
- Personal Protective Equipment, 29 CFR 1910.1030(d)(3): Employers shall provide appropriate personal protective equipment to employees when there is potential for occupational exposure. Employers responsibilities in providing such equipment include the following: ensuring employee use of the appropriate equipment; ensuring accessibility to the equipment; cleaning, laundering, and disposal of equipment; repair and replacement as needed to maintain its effectiveness; and, providing designated equipment removal and storage areas.

Transfer of Biological Agents

Transfer refers to the process of exchanging materials between facilities (USDHHS 1999). Regulations concerning transfer of select agents are intended to ensure the change in possession of select materials is within the best interests of the public and the nation. Security in transfer is achieved by the following:

- Justification of need for the select agent in the transfer process, and subsequent approval of the transfer process by a federal authority;
- Documentation of personnel and facilities involved in the transfer; and,
- Notice of delivery.

Justification and Approval

In accordance with 42 CFR Part 73, NIH and CDC require completion of CDC Form EA-101 for transfer of any select agent. Completion of the form is intended to provide adequate justification for the transfer, and acquire all necessary approvals. Specific information required by CDC Form EA-101 includes:

- Name of requestor and requesting facility;
- Name of transferor an transferring facility;

- Names of responsible facility officials for both transferor and requestor;
- Facility registrations numbers;
- Name of the agent(s) being shipped;
- Proposed use of the agent(s); and,
- Quantities (number of containers and amount per container) of agent(s) being shipped.

Personnel and Facility Documentation

CDC Form EA-101, as discussed above, requires of NIH employees requesting and transferring select agents, the officials responsible for approving those requests, and the facilities receiving and transferring the material.

Notice of Delivery

In accordance with *42 CFR Part 72/RIN 0905-AE70, CDC* requires notice of delivery of materials known to contain etiologic agents. Requesting facility responsible official must acknowledge receipt of the agent telephonically or otherwise electronically within 36 hours of receipt and provide a paper copy or facsimile transcript of receipt to the transferor within three business days of receipt of the agent.

Upon telephonic acknowledgment of receipt of the agent, the transferor shall provide a completed paper copy or facsimile of CDC Form EA-101 within 24 hours to registering entity in accordance with 72.6(c)(2) for filing in a centralized repository.

References

USDHHS 1998. U.S. Department of Health and Human Services, NIH Policy Manual, 3035 – Working Safely With Hazardous Biological Materials, Issuing Office: ORS/DS 496-2960. Release Date: 05/06/98.

IATA Dangerous Goods Regulations (DRG)

US Department of Transportation (49 CFR Parts 171-180)

US Department of Health and Human Services, Public Health Service (42 CFR Part 71: Foreign Quarantine, Part 71.54 Etiologic Agents, Hosts and Vectors); (42 CFR 72: Interstate Shipment of Etiologic Agents)

US Department of Agriculture, Animal Plant Health Inspection Service, National Center for Import and Export (7CFR 340); (9 CFR Parts 92, 94, 95, 96, 122 and 130)

US Department of Interior, U.S. Fish and Wildlife Service (50 CFR Part 14: IMPORTATION, EXPORTATION, AND TRANSPORTATION OF WILDLIFE)

US Department of Commerce, Export Administration Regulations (15 CFR chapter VII, subchapter C); (15 CFR Parts 730 to 799)

US Postal Service (39 CFR Part 111: Mailability of Etiologic Agents)

US Department of Labor, Occupational Safety and Health Administration (29 CFR Part 1910.1030)

APPENDIX D
Review of Biocontainment Laboratory Safety Record

Biosafety at National Institute of Allergy and Infectious Diseases:

1982-2003

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October 15, 2003

Biosafety at National Institute of Allergy and Infectious Diseases

1982-2003

The National Institutes of Health and the Centers for Disease Control (NIH/CDC) first promulgated National Guidelines for safe work with a broad range of infectious organisms in 1980. Four levels of physical containment and work practices were designated for agents with different virulence for humans and relative risk of infection from aerosols induced by laboratory manipulation. Biosafety Level 3 (BSL-3) is reserved for organisms that cause serious disease and which are known to be infectious via the respiratory route. Examples include *Mycobacterium tuberculosis* and West Nile virus. For such agents all procedures must be carried out in biosafety cabinets (BSC) fitted with high efficiency filters (HEPA). Centrifuges require sealed rotors so that aerosols that ensue if a tube breaks during spinning runs will be contained until the rotor is opened under the BSC. Air in such laboratories is maintained at negative pressure relative to hallways and cannot be blended with air to other laboratories and offices in order to prevent potential infection to others in the building. More and more such laboratories also have HEPA filters on laboratory room exhaust.

In addition to agents known to be aerosol transmitted, microbiological science continues to confront newly discovered viruses and bacteria for which aerosol infectiousness is uncertain. The NIAID has adopted a policy for such organisms that stipulates BSL-3 equipment and practices in BSL-2 laboratories with negative pressure. Work with the Human Immunodeficiency Virus (HIV) in the early 1980s led to adoption of that strategy for HIV and its close animal virus relatives, a policy that continues. Similar standards were initiated for work with hepatitis viruses at request of senior investigators, largely because new agents that cause hepatitis continue to emerge and little is known in early years regarding their infectiousness as aerosols.

This review is limited to work done during the past two decades by scientists at intramural laboratories of NIAID located on the Bethesda campus, at a neighboring facility in Rockville, MD, and at the institute's Rocky Mountain Laboratories in Hamilton, Montana.

Senior scientists were interviewed to ascertain agents studied, the variety of research programs that evolved over two decades, animals employed, if any, laboratory space, daily number of workers in the laboratories, and specific histories of laboratory accidents and consequences. Problems with function of facilities also were solicited and recorded.

Independent records of reported laboratory accidents that might expose workers to infection were reviewed. During the past 21 years all such accidents were to be reported quickly to the NIH Occupational Medical Service (OMS) for epidemiologic and medical evaluation as well as immediate prophylactic treatment if indicated. Invasive wounds in course of laboratory work and clinical care of persons with chronic HIV infection are of continuing concern. The OMS is now able to provide antiviral therapy within two hours of an accident on a 7day/24 hour basis when circumstances indicate the need for therapy.

Intake records of all accidents on the NIH campus were initially paper documents. Copies were forwarded to the Occupational Safety and Health Branch (OSHB) in the Director's office for to follow up circumstances of an accident and for remedial action when indicated. In addition to such immediate reaction to accidents and facility emergencies, the OSHB has developed standardized protocols for periodic review of all laboratories for compliance with NIH safety practices. Laboratories at BSL-3 level are reviewed at six month

intervals; all others annually. For the past decade, all records are computerized and electronic copies go from OMS to OSHB instantly. Records for this 21-year interval were cross-checked for details by staff of both Offices, together with specific scientist memory, in constructing the biosafety record for NIAID since 1982. Records for the Rocky Mountain Laboratories were reviewed with biosafety and scientific staff at that facility.

The detailed report is organized by Laboratory within the NIAID Division of Intramural Research. Agents, research agendas, containment levels, animal use, location and space for laboratories are presented in tabular form, together with histories of laboratory accidents and of facility problems that have affected work in those laboratories.

By any measure, the safety record at intramural NIAID laboratories, where work is done with the Institute's most pathogenic agents, is outstanding. No agent has escaped from any laboratory to cause infection in adjacent civilian communities. Indeed, this record stretches to almost 70 years at RML where several agents now on the national "Select List" have been studied for decades.

If one takes the number of 8-hour person days estimated by senior research staff during direct conversations and translates these into 2000 person hours per year in exposure to microbial organisms, impressive numbers emerge as shown in the following Table.

PERSONNEL HOURS WORKED AND OUTCOMES OF ACCIDENTAL EXPOSURES TO INFECTIOUS AGENTS: INTRAMURAL NIAID 1982-2003

HOURS AT RISK			
	BENCH	Animal	Total
BSL-3	553,000	81,500	634,500
BSL-2/3 P	2,235,500	360,200	2,555,200
Total	2,788,500	441,700	3,189,700

OUTCOMES OF ACCIDENTAL EXPOSURES			
	Clinical Infections	Silent Infections	Other Exposures, No infections
BSL-3	1	2	9*
BSL-2/3 P	0	2	15
Total	1	4	24

* One HIV invasive accident treated with anti-retroviral drugs. No infection ensued.

One clinical infection without sequelae and four silent infections in more than three million hours of exposure is a remarkable record, especially when continuous exposure of personnel to fluids containing HIV virus over many years is a significant part of that record. Indeed, only a single instance was considered worthy of immediate prophylaxis for that agent and no infection occurred.

Biosafety in NIAID laboratories demands, and receives, constant vigilance. I recommend, however, better documentation of communication between the OSHB and NIH Division of Engineering Services. I was unable to find very many records of specific facility problems and their outcomes. It might be well to have a brief computerized form for registry of each event that requires action, together with follow-up reports that find their way to OSHB.

Another concern is design and function of air handling systems for BSL-3 laboratories. In both Building 10 and the new Building 50, BSC IIB cabinets directly ventilated externally are an essential part of the overall exhaust system that always must be greater than the input air. If room negative pressure diminishes, the BSCs also shut down, a poor condition if aerosols are being generated in course of the work. Much better would be to have IIA BSCs as workstations. These would continue to capture aerosols regardless of overall room negativity. Hoods would not have to run continuously and room failure would not also release aerosols into the laboratory. The Uninterrupted Power Supply installed in Building 50 was a prudent decision. I hope that these questions will be/have been considered in the current renovation of Twinbrook III as BSL-3 laboratory. Finally, it was a pleasure to receive frank, careful responses from all the scientists I approached. They willingly turned from their particular microbial environments to candidly discuss the history of their work from a safety perspective.

This report is included in the Final Environmental Impact Statement of the Integrated Research Facility.

Biosafety at BSL-4

More than 20 Years Experience at Three Major Facilities

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October 15, 2003

Biosafety at BSL-4:
More than 20 Years Experience at Three Major Facilities

WHAT IS BSL-4, AND HOW DID WE GET THERE?

Special containment for work with infectious microbes in the United States originated during World War II in response to intelligence that the German army had a program for development of biological, in additional to chemical weapons that had been used during the first World conflict. Temporary facilities were established in a suburb of Frederick, Maryland, later to become the permanent Fort Detrick. During the 1950s and 1960s several agents, most notably the bacteria that cause plague and anthrax and the rickettsial organism that causes so-called Q fever, were produced in large quantities and in forms with properties that make highly infectious tiny particles in the air. The term used was, and is, 'weaponized.'

Infections among those working with these and other microbes were a recurrent problem. Under the inspired leadership of the late Dr. Arnold G. Wedum, recognized today throughout the world as the "Father of Biosafety," Fort Detrick borrowed technology from the nuclear industry to prevent such infections, especially those induced by small aerosols that arose during the course of routine laboratory manipulations. Stainless steel cabinets (termed Class III) were constructed and assembled in continuous airtight lines. Each had at least one pair of sealed glove ports to allow manipulation of hazardous materials in a sealed-off environment. Incubators, microscopes, and doors leading directly to autoclaves and to animal cabinets were integral to the cabinet line. The cabinets had a constant supply of filtered air and filtered exhaust fans to remove any particles generated during the work sessions. Air pressure in cabinet lines was negative to the laboratory room and the exhaust was filtered. The room itself also was negative to the rest of the building, and exhaust air was filtered before release to the environment. Thus workers, others in the building, and the outside community, were all protected against aerosol infection from agents otherwise intended for battle.

During these same two decades, new organisms with serious human pathogenicity were discovered in nature on several continents. Most of these, all of which were viruses, caused a syndrome (with variations) known as acute viral hemorrhagic fever (VHF). There was no specific treatment or vaccine available for any of them, except for the classical virus that causes yellow fever. That disease is now recognized as the prototype of VHF. Even more disturbing was the fact that aerosols were infectious for laboratory staff for most of these agents. Virology at Fort Detrick quickly entered the Class III cabinet habitat.

The recognition of Marburg virus in 1967 propelled the Centers for Disease Control (CDC) into this arena. That agency was asked to help with field studies designed to uncover the African reservoir for the virus, and it was decided that diagnostic reagents were needed. Visions of travelers returning from parts of the globe endemic for HF agents became a chronic concern. A small Class III cabinet laboratory was established in 1970 at the CDC. It had about 70 linear feet of cabinet line and a staff of two persons who tested samples from wild animals for infection and made diagnostic reagents for Marburg and other viruses of concern.

One year previously (1969), President Richard Nixon unilaterally terminated the national program of offensive biowarfare at Fort Detrick. Most of the buildings were given over to the National Cancer Institute. But the Army now expanded its defensive program. A new facility was constructed that became the principal laboratory of the U. S. Army Medical Research Institute for Infectious Diseases (USAMRIID). It opened in early 1971 with a mission to develop technology for detection and identification of potential biowarfare agents, to understand pathogenesis of the new VHF agents, to search for specific antiviral therapies, and to develop vaccines.

Another VHF agent, Lassa virus, appeared in Nigeria in 1969. When Marburg virus attacked two young Australians traveling in southern Africa in 1975, CDC Director David Sencer decided that it was time to

reinforce the nascent Special Pathogens Branch. A surplus large trailer was obtained from NIH and outfitted as a new laboratory for work with VHF agents. It had a Class III cabinet line. Space previously used as offices was redesigned as the first completely suited laboratory and animal room. Workers wore special positive pressured suits that could be hooked up to hoses from the ceiling that provided clean breathing air. Suits came in several sizes and each worker was now able to have gloves that truly fit their hands. All work was to be done in movable Class II laminar flow biosafety cabinets (BSC) that pulled air across the work surface then filtered it, with about half recirculated in the box and the rest released into the laboratory. Similar filtered enclosures were employed to house infected animals. Laboratory exhaust air was twice filtered before release to the environment, all solid wastes were autoclaved in double-door machines installed through a laboratory wall, and all liquid wastes were pressure cooked at high temperature before cool down and released to sanitary sewers. Workers leaving the laboratory stood in a chemical shower to decontaminate the "space" suits before doffing scrub suits and showering before leaving the facility. Various alarms and redundant systems were installed to ensure that power, continuous negative pressure, and breathing air were always available in emergency. Needles and scalpels were used as infrequently as possible and plastic ware replaced glass for almost all procedures.

The new CDC laboratory was opened at the end of 1978. Laboratories utilizing positive pressure suits also were ready at USAMRIID within months. These configurations allowed convenient installation and maintenance of new instruments and other equipment that was being developed for molecular work on viruses. The principles of biocontainment were: (1) capture each small particulate aerosol immediately where it is generated, (2) ensure that workers have functional hands, life support, minimum exposure to invasive accidents, and ready access to the tools required for research, and (3) make sure that systems for prevention of escape of aerosolized viruses to the environment are redundant. The BSC cabinets were the primary containment, the exhaust-filtered laboratories were the secondary, and even these were redundant.

By 1976, some leading molecular microbiologists became worried that new technology could potentially create novel organisms that might conceivably become Andromeda strains. The Director of the National Institutes of Health (NIH) ordered new guidelines for standards of microbiological safety for diverse agents with known properties of human pathogenicity and modes of transmission, as well as for newly discovered agents. The first edition of the NIH/CDC guidelines was published in 1980. Most work could be done in ordinary laboratories at BioSafety Level 2 (BSL-2). Others that cause more serious illness in humans, and/or for which no treatment is available, were assigned to BSL-3. All work was to be done in Class II biosafety cabinets. Room air was to be under negative pressure relative to hallways with no recirculation to other space in the building.

BSL-4 was reserved for VHF agents, certain tick-borne encephalitis viruses, and a simian herpesvirus for which human infection is almost universally fatal. At the time, this meant USAMRIID and CDC Special Pathogens, but authorities in South Africa were progressively concerned about VHF on their continent. Ebola virus, an even more virulent relative of Marburg, had been discovered in 1976. Rift Valley fever virus had caused its first-ever epidemic that included hemorrhagic fever. Crimean-Congo virus was a new concern. To meet these challenges, a BSL-4 laboratory, modeled on the Detrick and Atlanta prototypes, was constructed outside Johannesburg and commissioned in 1980. It had both suit and cabinet-line laboratories.

These three laboratories were virtually *the* sites of BSL-4 viral work during the past 22-30 years. With experience over time, most investigators chose to work primarily in the positive-pressure suit environment. Indeed, at the end of the1980s, CDC moved into new large laboratories that were almost devoid of Class III cabinet lines. Moreover, the Johannesburg laboratory, now part of the National Institute for Communicable Diseases (NICD), recently removed its Class III cabinets in order to expand positive-pressure suit space. Only the British BSL-4 laboratories continue to depend on Class III cabinet line configurations. All recently constructed Level 4 facilities in other countries, as well as those proposed for ours, are positive-pressure suit labs. Accordingly, this review will not include biosafety at the Porton Down facility. We are concerned principally with the track record of, and a risk analysis for, BSL-4 positive-pressure suit laboratories.

That record is exemplary. Most individuals who begin work in BSL-4 suites are already experienced microbiologists. Specific training for use of the positive-pressure suits and for safe execution of all procedures is standard practice at all of the laboratories. In context of current international concern regarding potential use of some of these viruses as weapons of terror, access to the facilities and to individual laboratories is carefully controlled. At two of the facilities in the United States individual security clearance is required to qualify for work at the BSL-4 level. The viruses under study do not escape, neither by accident nor by covert design. Reviews of individual facilities are summarized below.

USAMRIID — 1972-2003

Persons Interviewed:

Drs. Peter Jahrling, Chief Civilian Scientist; Gerald Eddy, retired Chief, Virology Division.

Research Program:

Pathogenesis of viral infections in animal models, including clinical and anatomical pathology. Quantitative susceptibility of animals to aerosol infection by VHF pathogens. Development of diagnostic assays and air sampling detectors. Molecular anatomy and genetics of agents. Drug screening program in search of anti-viral compounds. Development of live attenuated, inactivated, and recombinant vaccines.

Agents Studied:

Machupo, Junin, Guanarito, Sabia, and Lassa arenaviruses; Marburg and Ebola; Rift Valley fever and Crimean-Congo hemorrhagic fever viruses; Tick-Borne encephalitis virus. *Yersinia pestis* and *Bacillus anthracis.*

Animals Used:

Mice, hamsters, guinea pigs, non-human primates, wild rodents, lambs,

Site:

Two buildings, Fort Detrick, Maryland. Total BSL-4 space: about 6500 sf. One third is animal space and suit/cabinet ratio of lab space is about 2:1.

Time Devoted in BSL-4 Space:

Approximately 343,980 hours. (6.5 persons/8 hour day x 1680 hours/year x 31.5 years).

Laboratory Accidents and Outcomes:

During early years when work was completely in cabinets, invasive accidents resulted in treatment with human plasma containing specific antibodies to virus in question, as well as confinement in an isolation suite in one building that was also set up as an intensive care facility in event that a worker became ill after accidental exposure to an agent. Two invasive accidents were of most concern:

November 1979. Accidental finger puncture with needle on a syringe loaded with Lassa virus. Ribavirin and immune plasma were given. (This was an experimental therapy for monkeys under development at the Institute.) No illness or serological evidence for infection occurred.

December 1982. During autopsy, a bone fragment of a monkey infected with Junin virus punctured a finger. Immune plasma was used and no clinical or subclinical infection ensued.

CDC SPECIAL PATHOGENS

Persons Interviewed: Senior Scientists and Author

Research Program:

Development of diagnostic methods and reagents for diagnosis of all BSL-4 agents. Pathogenesis of viral infections in animal models, including natural wild reservoirs. Molecular anatomy and genetics of VHF agents. Limited vaccine development work. Response to VHF epidemics in natural settings. Diagnosis, clinical pathology and virology, discovery of new agents.

Agents Studied:

Five arenaviruses, Marburg, Ebola, Crimean-Congo HF virus, Rift Valley fever virus, Nipah and Hendra viruses, Russian spring summer encephalitis and Tick-Borne encephalitis viruses, Omsk and Kyasanur Forest disease viruses, Hantavirus (animal work only).

Animals Employed:

Mice, hamsters, guinea pigs, non-human primates, rats, five wild rodent species for rodent-borne agents.

Sites:

Building A: 1970-78. About 70 linear feet of Cabinet line.

Building B: 1979-1989. About 900 sf with 30 ft cabinet line, 300 sf positive-pressure suit lab and 200 sf of positive-pressure suit animal space.

Building C: 1990-2003. About 5000 sf of which approximately 30% is animal space. Laboratory is entirely positive-pressure suit operated.

Time Devoted in BSL-4 Space:

120,560 hours.

Laboratory Accidents and Outcomes:

Animal bite; Hantavirus infected rodent, no infection.

Animal bite; animals being inoculated with Hantavirus. Pre-inoculation bite from rat.

Needle stick to worker prior to setting up an inoculum with mouse-adapted Ebola virus. No infection.

Autoclave door interlock failed and a load not autoclaved was opened, but not handled. No infections resulted.

Multiple events over the years of outer gloves or suits developing tears or holes detected during work. Such incidents are evaluated and followed up. No treatments were ever used and no infections resulted.

Facility/System Failures: None of note that caused interruption of work.

National Institute for Communicable Diseases

Johannesburg, South Africa, 1980-2003

Person Interviewed:

Dr. Robert Swanepoel, BSL-4 Laboratory Director

Research Program:

Diagnostic reagents and support for all HF outbreaks in Africa and neighboring regions when requested,; pathogenesis of infections in animals, especially candidate wild reservoir species; clinical virology; molecular biology of selected hemorrhagic fever viruses; field investigations of natural history of disease outbreaks; and seroepidemiology of infections in humans and animals.

Agents Studied:

Marburg and Ebola viruses, Rift Valley fever virus, Crimean-Congo HF virus, ten hantaviruses.

Animals Employed:

Mice, guinea pigs, rabbits, bats, tortoises, pigeons, snakes, roaches, spiders, frogs, millipedes, snails, 20 species of wild rodents, hares, hedgehogs, guinea fowl, chickens, etc. Much animal work was devoted to a search for wild reservoirs of Marburg and Ebola viruses.

Site:

Rietfontein, 4500 sf. Space divided into 721 sf positive-pressure suit lab and 222 sf similar animal holding room, plus cabinet lab of 999 sf (now defunct). Remaining 1443 sf devoted to change rooms, showers, and service corridors.

Time Devoted in BSL-4 Space:

Approximately 40,000 hours in nearly 23 years.

Laboratory Accidents and Outcomes:

Bat bite through double gloves. No infection.

Multiple other accidents. Those exposed are monitored closely for 21 days, during which time they are not permitted to leave town—as are all employees after their last day of work inside BSL-4 space. No infections recorded.

Facility/System Failures:

Only one that caused shutdown of operations. About 5 liters of highly concentrated Marburg virus was suddenly aerosolized when worker opened chamber to add a bit more fluid without closing the nitrogen pressure tank and bleeding off pressure. Laboratory was mopped for several hours with glutaraldehyde, and finally decontaminated with formaldehyde gas. No infection occurred in two "exposed" workers. There was no breach in BSL-4 containment, and no infections occurred in neighboring open-air monkey colonies on the campus. This was a maximum challenge to BSL-4 containment, and I am aware of no other event remotely comparable in terms of concentration and volume of a highly lethal virus.

Summary

No clinical infections occurred at three institutions during work with BSL-4 agents, mostly hemorrhagic fever viruses during the past 31 years. Almost half a million hours of laboratory (and field) exposure have been recorded, the majority of which was time spent in positive pressure suits. Nor have there been major defects or incidents in operation of the physical facilities. No escape of any agent with clinical consequences for neighboring communities occurred.

Invasive injuries were infrequent, eloquent testimony to the awareness of the dangers and the daily care observed by workers who volunteer for such duty. One laboratory inadvertently carried out a maximum aerosol challenge to BSL-4 containment with a highly pathogenic hemorrhagic fever virus. Virus did not escape the laboratory, nor was a worker infected.

The zero numerator of infections in these three laboratories and the huge denominator of exposure hours make it impossible to provide a number for 'risk of infection' to either laboratory workers or outside communities. Nevertheless, that number must be small. When the value of diagnosis, treatment, and control of deadly outbreaks of hemorrhagic fever over the past three decades is added to this equation, risk/benefit clearly comes out in favor of continued operation of BSL-4 laboratories.

Indeed, considering new challenges posed to the world community by these agents, it is fair to conclude that more such facilities are needed. Better therapeutic agents are desperately needed. High priority also must go to the development of vaccines that can protect laboratory and hospital personnel in countries where natural epidemics occur, as well as first responders to intentional aerosol attack on any community.

This report is included in the Final Environmental Impact Statement of the Integrated Research Facility.

ROCKY MOUNTAIN LABORATORY (RML) Safety Record

Persons Interviewed: Ted Hackstadt, John Portis, Bruce Cheesbro, Tom Schwan, Mike Parnell, Cliff Barry, Joe Hinnebusch, Jim Musser, Mort Peacock

BSL-3 Agents: *Coxiella burnetti, Rickettsia rickettsii, Chlamydia trachomatis*						
Period	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Exposures/Infections/Remedies to Infectious Agents**	**BiosafetyFacility Systems, Problems & Remedies**
1982-1988	Pathogenesis, cell biology, immunology, genetics of distinct strains of agents. Recombinant antigen development, vaccine development.	Guinea pigs, mice, one experiment with *C. burnetti* in dogs.	Bldg 16 East room, bench 800 sf. Bldg 16, East, animal 300 sf.	Bench 5.3 Animal 2.0	None	Heating in Bldg 16 poor. Labs extremely cold in winter. Pumps sometimes failed so lack of water threatened animal health. Exhaust ventilation in Bldg 16 failed several times during 1980s. Staff was not immunizaed against *C. burnetti* so repeated ventilation problems caused sealing of animal room from the laboratory area in 1988. Animal studies dropped for several years.

BSL-3 Agents: *Coxiella burnetti, Rickettsia rickettsii, Chlamydia trachomatis*						
Period	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Exposures/Infections/Remedies to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
1990-2002	Work continued on pathogenesis, genetics, and biology of organisms in cells.	None	Bldg 16 East room, bench 800 sf.	Bench 10.0	1998: Research fellow hospitalized for pneumonia in left lung. *C trachomatis* isolated and specific sero-conversion was documented. Uneventful recovery with antibiotic therapy. Researcher did sonication of cultured organism in Class II Biosafety Cabinet (BSC) two days prior to onset of illness. Three large scale purifications of organisms done during 3 week period before illness. The specific source of infection remained indeterminant. All procedures were reviewed with staff. Particle masks were adopted for all aerosol-generating procedures and for 30 minutes after completion of these. Centifuge rotors were to be opened only in BSC and both the instrument and rotors were to be examined for leaks after each run. Alcohol-soaked sponges were required to surround the sonication tube and all worker faces were to stay outside the glass front of the BSC. No further infections since that time.	No new problems.
2002-2003	Resumption of full program including use of animals for ricksettsial agents. Virulent strains will be utilized.	Guinea pigs and mice.	Bldg. 25 bench 560 sf; Animals (see Biosafety column.)	Bench 6.0 Animal (see Biosafety column.)	None	Beginning in 2002, BSL-3 animal work for all agents at RML was transferred to the new building 25. Space devoted to animals is 1000 sf. Animal species so far housed are mice, guinea pigs, and non-human primates. Persons per day for operation are 2.0.

BLS-3 Agent: *Mycobacterium tuberculosis*						
Period	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Exposures/Infections/Remedies to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
1993-2001	Genetics and immunology. No drug-resistant strains utilized. Mutants were generated. Lipid and protein content as weel as replication dynamics of these mutants were markers utilized to determine relative virulence. Electron microscopy of human tissues.	Mice. Limited experiments with wild strains.	Bldg HD-2 Bench 540 sf; Animal 580 sf. Bldg 5 Electron microscopy ~400 sf.	Bench 7.0 Animal 2.0	1996: Laboratory technician converted PPD skin test for the agent. There was no history of an obvious invasive accident or any apparent breakdown in laboratory procedures to prevent aerosols. Worker treated for 6 months with isoniazid. No pulmonary or other evidence of disease occurred. Major modifications instituted in equipment and procedures to prevent aerosol infection: (1) New high-speed sealed centrifuge rotor. (2) Large plastic bottles as visable secondary containment for roller bottles. (3) Upgraded NIOSH-approved face masks. (4) Additional UV fixture for dirty dress-out chamber. (5) Autoclave treatment for ice buckets leaving lab. Relocation of ice machine inside the laboratory. (6) Chest xrays each six months; all personnel, even those PPD positive when initially employed in lab.	Exhaust fan broke in HD-2 and BSC also failed. A winter pipe break in same building flooded the laboratory. Research program moved to Twinbrook II, Rockville, MD, in 1998. Electron microscopy with outside collaborators continues.

BLS-3 Agent: *Mycobacterium tuberculosis*						
Period	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Exposures/Infections/Remedies to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
1993-2001	*M tuberculosis* exposures continued.				2000: PPD skintest conversion. Employee treated with isoniazid. No clinical or radiological evidence for disease ensued. Employee worked in the Electronic Microscopy Branch (EMB) in Bldg 5. Work involved prep of samples submitted by outside collaborators in EM. Centrifugation done outside BSC. Although all samples supposedly were inactived before receipt at RML, suspicion is that residual live bacteria were source of infection. Several modifications to equipment and procedures instituted; (1) A modern sealed centrifuge was installed. (2) Bldg 5 air handling was upgraded and alarms for BSC function were provided. (3) Documented inactivation protocols and safety tests must now accompany materials received from outside sources. (5) All samples to be processed as though they still contain viable organisms.	
2002-2003	Global genetic analysis using microarray technology for different strains of bacteria. No drug resistant strains in use.	None	Bldg. 25 Bench 560sf	Bench 1.5	None	None

BSL-3 Agent *Yersinia pestis*						
Period	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Exposures/Infections/Remedies to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
2002-2003	Interactive cell and animal biology of bacterial strains and mutants in mice and fleas.	Mice, fleas	Bldg 25 Bench 560 sf Animal (see above)	Bench 6.0 Animals (see above)	None	None
BSL-3 Agent Transmissible Spongiform Encephalopathy (TSE)						
2022-2003	Prion genetics and studies on transmissibility of strains from different animal species.	Mice, non-human primates	Bldg 25 Bench 560 sf Animal (see above)	Bench 6.0 Animal (see above)	None	Work with TSE agent causing scrapie antedates formulation of national guidelines and was done less than BSL-2 containment. Recent program initiated after certification of new BSL-3 labs in Bldg. 2. 5
BSL-2/3 Practices Agent *Yersinia pestis*						
Date	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Accidental Exposures to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
1986-2000	Rapid detection of agent, PCR technology used when available. Establishment of flea colonies. DNA probe developed, fraction I antigen cloned. Most work with avirulent	Mice, fleas.	Bldg 12, Bench 100 sf; Animal 100 sf. Bldg 13, Insectary 125 sf.	Bench 2.0 Animal 1.2	None	None

	bacterial strains. Few experiments with virulent bacteria for immunization studies.					
2000-2002	Continued	Mice, fleas	Bldg 5, Rm 5203 Bench, 600 sf. Bldg 12, animal, 100 sf. Bldg 13, Insectary, 125sf	Bench 4.0 Animal 0.2	2001: An open container of *Y. pestis* fell off a shaker during the night. Several workers entered the lab next morning and the accident was immediately discovered. Surfaces were decontaminated and lab was closed until a new BSL-3 was available. No infections occurred.	None
BSL 2/3 Practices Agent: Lentiviruses						
Date	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Accidental Exposures to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
1987-1997	Cell culture work for drug screening. Molecular biology of HIV.	None	Bldg 5 Bench 300 sf.	Bench 1.5	1987: Worker punctured finger on broken cover slip in HIV lab. No medical treatment. No infection occurred.	None
1998-2001	No change	None	Bldg 10 Bench 300 sf.	Bench 1.5	None	None
2002-2003	Current program includes cell culture assay. Insertion of co-receptors into HeLa cells gives stainable virus plaques in 3 days. Work includes cloning, tests for differential cell susceptibility, and creation of pseudotype HIV/SIV viruses with	Mice	Bldg 3 Bench 600 sf. Bldg 13 Animal 150 sf.	Bench 1.5 Animal 0.5	None No needles used in work; no significant concentration of virus is done. Personnel tested for HIV antibodies annually.	None

	a few experiments in mice. Transgenic rats will be used in future.					
BSL 2/3 Practices Agent: *Chlamydia trachomatis*						
Date	**Research Program**	**Animals Utilized**	**Location**	**Avg Persons/ Day**	**Accidental Exposures to Infectious Agents**	**Biosafety Facility Systems, Problems & Remedies**
2002-2003	Basic pathogenesis. Antigens as vaccine candidates.	Mice, non-human primates	Bldg 1 Rms 1201 and 1202 Bldg 2 Rms 2204, 2206, 2208. Bench 2102 sf. Bldg 13 Animal ~300 sf (shared)	Bench 13 Animal 0.5	None	None

APPENDIX E
Standard Operating Procedures for a BSL-4 Facility

The following documents are the standard operating procedures used in Biosafety Level 4 laboratories at the NIH facility in Bethesda, Maryland. Standard procedures for the Integrated Research Facility would be written if the decision is made to select the proposed action. Those standard operating procedures would be similar to these, covering the same subjects with the same amount of detail. In most cases, the procedures would be the same, unless different equipment would be used.

Part 1 is the Standard Operating Procedures.

Part 2 is Decontamination Equipment and Procedures.

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ACRONYMS

AAALAC	**American Association for Accreditation of Laboratory Animal Care**
AALAS	**American Association for Laboratory Animal Science**
ACUC	**Animal Care and Use Committee**
ARAC	**Animal Research Advisory Committee**
BMBL	**Biosafety in Microbiological and Biomedical Laboratories**
BSC	**Biological Safety Cabinet**
BSL	**Biosafety Level**
CDC	**Centers for Disease Control and Prevention**
DES	**Division of Engineering Services**
DOT	**Department of Transportation**
DS	**Division of Safety**
HEPA	**High Efficiency Particulate Air**
IBC	**Institutional Biosafety Committee, NIH**
ICD	**Institute/ Center/ Division**
ILAR	**Institute of Laboratory Animal Resources**
IPM	**Integrated Pest Management**
MCL	**Maximum Containment Laboratory**
MPW	**Medical Pathological Waste**
NIH	**National Institutes of Health**
NRC	**Nuclear Regulatory Commission**
OD OIR	**Office of the Director, Office of Intramural Research**
OMS	**Occupational Medical Service**
OSHA	**Occupational Safety and Health Administration**
OSHB	**Occupational Safety and Health Branch**
PI	**Principal Investigator**
PHS	**Public Health Service**
PRC	**Policy Review Committee, NIH**
RSB	**Radiation Safety Branch**
USDA	**United States Department of Agriculture**
USFWS	**United States Fish and Wildlife Service**
VHP	**Vapor Phase Hydrogen Peroxide**

Part 1 – Standard Operting Procedures

1.0 INTRODUCTION

1.1 PURPOSE

Operational and safety procedures for the Biosafety Level Four (BL-4) Laboratory (also referred to as the Maximum Containment Laboratory [MCL]), Building 41A, National Institutes of Health (NIH), are contained within this manual. Three laboratory/animal suites are housed within the unit. In some circumstances, processing BL-3 agents may require BL-4 containment. Most of these BL-4 pathogens are highly virulent for humans and infectious by the aerosol route. They often are capable of direct transmission from person to person; and produce diseases for which there is no specific treatment or prevention available.

1.2 POLICY STATEMENT

Operational and safety procedures described in this manual shall apply to all program and support personnel associated with the facility. Modifications to these procedures or to the facility shall not be made without written approval of the NIH Institutional Biosafety Committee (IBC), Policy Review Committee (PRC) and the Chief, Occupational Safety and Health Branch (OSHB).

NO PERSON SHALL WORK IN THE MCL WITHOUT HAVING READ THIS MANUAL AND ATTENDED REQUIRED TRAINING SESSIONS CONDUCTED BOTH BY THE PRINCIPAL INVESTIGATOR AND THE MCL FACILITY MANAGER AND/OR THE MCL OCCUPATIONAL SAFETY AND HEALTH SPECIALIST, OF BUILDING 41A.

NO PERSON SHALL WORK ALONE IN THE MCL AT ANY TIME.

THE RESPONSIBILITY FOR MAINTAINING A CLEAN LABORATORY ENVIRONMENT REMAINS WITH ALL INDIVIDUALS WHO WORK IN THE MCL. THE RESPONSIBILITY FOR HOUSEKEEPING IN THE MCL IS PART OF DAILY OPERATING PROCEDURES.

Use of the MCL shall be limited to programs specifically approved by the NIH IBC, PRC and the Chief, OSHB.

1.3 RESPONSIBILITIES AND AUTHORITY

1.3.1 Chief, Occupational Safety and Health Branch

The MCL shall be the direct responsibility of the Chief, OSHB, NIH, who shall ensure that the procedures in this manual are followed at all times. The Chief may delegate authority for conducting specific programs in the MCL to other appropriate personnel as necessary.

1.3.2 Principal Investigator (PI)

The PI shall have direct responsibility for conducting the research in a manner that minimizes risks in the MCL. He/she has responsibility for the following:

Approval: Obtaining the necessary approval from the NIH IBC and PRC for the research program prior to the commencement of work in the MCL. The PI shall develop the animal protocol, and coordinate that protocol with the ICD veterinarian and the MCL Occupational Safety and Health Specialist.

Biohazards: Ensuring that program and support personnel (prior to working in the MCL) (i) are aware of biohazards and precautions to be taken in conducting the research program; (ii) are advised of the nature and

assessment of the real and potential biohazards and (iii) are informed of the indicators of accidental infections.

Medical Surveillance: Recommending appropriate (i) immunizations, (ii) serologic monitoring, (iii) other medical monitoring and (iv) post exposure prophylaxis.

Medical Clearance: Obtaining medical clearance for all employees from OMS - within the three months - prior to training in the MCL. All immunizations/testing shall be updated at that time for each employee.

Training: Instructing and training the program staff in the practices and techniques required for the safe conduct of the research program and the operation of the MCL.

Supervision: Supervising the program staff to ensure that their performance complies with the required standards of safety in the MCL.

Emergencies: Preparing, in collaboration with the Facility Manager, procedures for dealing with accidental spills and overt exposures among program personnel. Reporting to the Chief, OSHB, the Medical Director, OMS and the Facility Manager, problems pertaining to the (i) exposure of personnel, (ii) compromise of biological or physical barriers or (iii) major equipment failure which could compromise safe operations in the MCL (Section 2.2.5). Contacting any employee with an unexplained work absence. The employee shall be contacted by 10:00 a.m. on the day of the unexplained absence. The PI shall seek the advice of the Chief, OSHB and the Medical Director, Occupational Medical Service (OMS).

Monitoring of Operations: Correcting procedures that may result in hazardous incidents or problems. Notifying the IBC, PRC and OSHB of (i) such modifications in program, (ii) new safety procedures and (iii) any unexpected experimental results, e.g. unexpected results in laboratory experiments.

1.3.3 Facility Manager

The Facility Manager of the MCL is directly responsible to the Chief, OSHB, or his/her designee, and shall supervise the day-to-day operations of the MCL. The Facility Manager has the responsibility for the following.

1. Emergencies: Notifying the Chief, OSHB, immediately, of any incident or problem that compromises the safety of the staff or the integrity of the MCL.
2. Training: Training in collaboration with the PI and with the assistance of the MCL Occupational Safety and Health Specialist in the required training program for new staff or visiting scientists designated by the Chief, OSHB.
3. Decontamination: Supervising (i) decontamination procedures for all equipment or other material which leaves the MCL, and (ii) the annual gaseous decontamination of the MCL with the assistance of the MCL Occupational Safety and Health Specialist.

1.3.4 MCL Occupational Safety and Health Specialist

The Occupational Safety and Health Specialist is responsible for the following:

1. Assist the PI with research projects in the MCL.
2. Ensuring that physical containment systems, support equipment, waste disposal, and operation of the MCL are in accordance with the design of the MCL.
3. Ensuring that MCL maintenance procedures are conducted in a manner that precludes hazard to personnel and preserves the integrity of the MCL.
4. Acting as liaison with the staff for engineering and safety support systems. In the event that the MCL Occupational Safety and Health Specialist is unable to attend to these duties, the designated personnel of the OSHB staff will take on these duties on a rotating basis.
5. Assisting with the required training program for new staff, visiting scientists, and animal care personnel designated by the Chief, OSHB.

6. Decontamination procedures for equipment or other material before it leaves the MCL, and the annual decontamination of the MCL.
7. Perform daily Critical Systems checklist for the MCL.

1.3.5 Other Personnel Working in the MCL

1. All persons working in the MCL shall comply with the policies and practices established by the NIH IBC, PRC, the Chief, OSHB and the PI.
2. All personnel working in the MCL shall follow all safety practices and procedures required by good laboratory practice, the CDC and NIH publication entitled *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) and OSHA.
3. They shall report to the Facility Manager and/or Occupational Safety and Health Specialist any event that may (i) constitute potential exposure, (ii) result in creation of a potential hazard, (iii) impair safe operations in the MCL.
4. An employee who becomes ill with any febrile illness shall immediately notify his/her supervisor.

1.3.6 Policy Review Committee

The PRC shall be appointed by the Deputy Director for Intramural Research (OD DIR), NIH. The PRC shall consist of a member of the NIH IBC; the Chief, OSHB; a member of the ICD Animal Care and Use Committee (ACUC); ICD Scientific Director of the appropriate Institute; and accredited members may be requested to participate on an ad hoc basis. Non-NIH personnel may be requested to participate depending on the particular research under consideration. The functions of the committee are to (i) the PRC shall assess the program relevance, policy aspects and priority of the proposed studies; (ii) advise on the use of vaccinations as additional protection for MCL staff (with consultation with OMS); (iii) evaluate potential methods of treatment or post-exposure prophylaxis that could be applied to MCL personnel and (iv) advise in the evaluation and management of potential MCL exposures.

1.3.7 Application Procedures

Intramural applications will be reviewed in the following order: (i) Laboratory/Branch Chief, (ii) Scientific Director of the Institute, (iii) the NIH IBC, (iv) the ACUC, if required and then (v) the PRC.

Extramural applications will generally be reviewed in the following order: (i) the relevant ICD program officer, (ii) the NIH IBC, (iii) the ACUC, if required, (iv) the PRC, and then (v) results of these reviews will be returned to the sponsoring ICD for implementation. Each extramural application will have an intramural collaborator identified by the Scientific Director of the appropriate ICD.

All applications will be required to include a detailed protocol, which will be reviewed on the basis of scientific merit and biosafety standards.

The PI is responsible for completing the following forms:

1. A "Request to Use the Maximum Containment Laboratory Checklist" (Appendix A) and an "Authorization for Entry into the MCL" (Appendix B) must be signed and approved by the NIH IBC, PRC, OSHB and OMS before work is initiated.
2. All users of radioactive materials must first attend and successfully complete the "Radiation Safety in the Laboratory" course and register with the Radiation Safety Branch (RSB).
3. A Registration Of Materials (Potentially) Pathogenic to Humans document (Appendix R) and an Animal Study Proposal, shall be submitted to the NIH IBC, the ACUC and PRC, if applicable.

2.0 OPERATIONS AND SAFETY PROCEDURES

2.1 EMPLOYEE TRAINING AND ORIENTATION

Training, unique to the MCL, is provided by the PI and OSHB. Training requirements are listed on the Training Checklist for Authorization to Work in the MCL (Appendix D). The form must be signed by the Facility Manager or the Occupational Safety and Health Specialist upon completion of the checklist activities, and placed in the Facility Core Center file. A copy shall also be kept by OSHB. The training will include:

1. Formal safety briefing by OSHB, including training and orientation in MCL techniques and procedures
2. Reading the MCL Safety and Operations Manual
3. Briefing on the Entry and Exit Procedures (Section 2.2.4 and Section 2.2.3)
4. Attending the NIH courses on Laboratory Safety and HIV and other Bloodborne Pathogens.

2.2 EMPLOYEE SAFETY

2.2.1 Personal Protection Equipment

The primary source of protection for personnel working in the MCL is the *CHEMTURION* encapsulating positive pressure suit (Appendix E).

Positive air pressure is maintained by umbilical-fed air which is supplied to the suit through the air inlet manifold assembly fitted with a HEPA filter. This filter is located on the right front torso and prevents intake of potentially contaminated air from the laboratory. Air hoses are located throughout the MCL.

2.2.2 MCL Access

Access is limited to staff members and individuals who have a work-related need to be in the MCL. Access requires the approval of the IBC, PRC and Chief, OSHB. Building 41A entry involves the use of a proximity card. Proximity cards shall be issued by the Facility Manager. A proximity card is a controlled item to be used only by the person to whom it is issued and must never be loaned. A floor plan of Building 41A showing the proximity card readers is in Section 5.1.

A biohazard warning sign, incorporating the universal biohazard symbol, will be placed on the interior MCL entry door of Building 41A.

2.2.3 Entry Procedures

Each day, prior to entry of personnel into the MCL, the MCL Occupational Safety and Health Specialist shall perform a Critical Systems check (Appendix G) to ensure that the MCL is safe for operation. This inspection will specifically include proper functioning of the air handling system, processed waste system and breathing air system, as well as notation of any obvious problems with the other systems. This checklist shall be filed in the Facility Core Center. Laboratory personnel shall not enter the MCL until the checklist has been completed and signed. If the MCL Occupational Safety and Health Specialist cannot perform the Critical Systems check, the MCL Occupational Safety and Health Specialist shall notify the Chief, OSHB, or the Facility Manager. When such notification is given, an OSHB staff member, appointed by the Chief, OSHB, must make the Critical Systems check of Building 41A. All personnel shall sign-in daily on the Personnel Log Sheet (Appendix H) located in the Facility Core Center.

Personnel who plan to enter the MCL before 8:00 AM or exit after 6:00 PM on weekdays, or anytime on weekends or holidays, must notify the MCL Occupational Safety and Health Specialist (6-2346) and NIH security (6-5685). The MCL Occupational Safety and Health Specialist shall be notified 24 hours prior to that day.

On the weekends, it is the responsibility of the MCL scientific personnel who enter the MCL to coordinate with OSHB to have the Critical Systems check completed prior to entering the MCL.

Personnel must enter the MCL through the change rooms and decontamination shower. The sequence shall be reversed to exit. The procedure of donning a positive pressure suit requires the presence of a second individual to ensure complete sealing of the outer plastic closure. The procedures for entry are outlined in detail in Appendix F.

Any overt spills that contaminate the positive pressure suit should be decontaminated immediately through the use of disinfectants, which are located in multiple sites throughout the laboratory. Do not wait until the exit decontamination shower to tend to any possible contamination of the positive pressure suit.

2.2.4 Exit Procedures

Proceed to the boot area and remove the protective footwear and leave on the racks provided. Enter the decontamination shower/airlock and attach suit to an air line. Ensure both doors (with inflatable gaskets) are securely closed. For detailed operation of decontamination shower, see Section 3.3.8 and Appendix I.

2.2.5 Suit Malfunction

Suits are checked for air leaks prior to entry to the MCL as described in Appendix F. If a leak or rip is discovered while in the laboratory, DO NOT DISCONNECT the suit from air supply. Immediately disinfect the area around the hole and dry; then cover with tape (vinyl tape is stationed at several locations throughout each laboratory). EXIT THE LABORATORY AS SOON AS PRACTICAL. REPORT ANY BREACH OF SUIT INTEGRITY TO THE MCL OCCUPATIONAL SAFETY AND HEALTH SPECIALIST. Check major seams in the suit if the disposable jump suit contains wet spots after exiting decontamination shower. Contact the MCL Occupational Safety and Health Specialist if this occurs.

2.2.6 Spill Cleanup

Spills in the MCL involving infectious agents, radioisotopes, and chemicals must be dealt with promptly and in a manner to eliminate the hazard. All spills will be reported to the MCL Facility Manager and/or the MCL Occupational Safety and Health Specialist. Areas in which spills of potentially infectious materials occur will be thoroughly treated with a disinfectant solution to inactivate the biological agent. Small spills on counter tops or in biological safety cabinets will be wiped up and any absorbent materials used in the cleanup will be bagged and autoclaved. Larger spills on the floor will be disinfected and flushed with water into the floor drain. Decontaminate the area with an appropriate disinfectant. Minimize the volume of water used for the water rinse. All liquid waste in the MCL is automatically processed by heat treatment and then pH balanced prior to disposal.

Notify the MCL Staff (who will notify the Radiation Safety Branch) immediately of spills of radioactive material under the following circumstances:

1. a large activity (millicurie quantities) spilled,
2. a large volume (>1 liter) spilled,
3. a large area (>10 square feet) is contaminated,
4. personnel contamination or injury occurred.

Radioactive materials spill cleanup

1. Place absorbent material over the spill to keep it from spreading.
2. Notify others in the area, and limit access to the spill area.
3. Monitor yourself and others for contamination and decontaminate if you find any.

4. Label the boundaries of the spill area with "Caution Radioactive Material" tape.
5. Gather cleaning supplies such as moistened paper towels and scouring powder or any commercially-available detergent.
6. Minimize the volume of water used to decontaminate.
7. Begin cleaning at the edges of the spill and work towards the center (lowest to highest level of contamination).
8. Dispose of all cleanup materials in double plastic bags and label as radioactive waste, include the type of isotope, activity, date and your initials.
9. Re-survey yourself for contamination, including the bottom of boots, to ensure that no spreading of contamination has occurred.
10. After decontamination is complete, a thorough smear survey of the area should be completed to ensure that removable contamination is <2,200 dpm/100cm^2 in a restricted area and <220 dpm/100cm^2 in an unrestricted area.

If the spill also contains infectious material, label the bag with the infectious agent and treat the area with the appropriate dilution of disinfectant. The bagged radioactive material will be stored in a central radiation storage area and removed when appropriate.

2.2.7 Illness Notification

Any employee who becomes ill with any febrile illness shall immediately notify his/her supervisor. If the supervisor cannot be reached, the employee shall immediately notify OSHB and/or the Facility Manager of Building 41A. Phone and pager numbers are listed on the Illness Surveillance Notice (Appendix J) provided to each MCL employee.

2.3 RADIOACTIVE MATERIAL

2.3.1 Radioactive Isotope Authorization in the MCL

Authorized use of radioactivity in the MCL shall conform to the policies, practices, and guidelines set forth by the NIH Radiation Safety Branch (RSB) and The National Institutes of Health Radiation Safety Guide, in compliance with Rules and Regulations established by the United States Nuclear Regulatory Commission and outlined in "Standards for Protection Against Radiation" Title X, Code of Federal Regulations, Part 19, 20.

RSB shall be notified at least one month prior to use of radioactive materials in the MCL. A protocol shall be submitted to RSB for all work involving radioactive material. The removal of radioactive waste and all laboratory surveys shall be conducted as recommended by RSB.

2.3.2 Records

KEEP A RECORD OF ALL RADIOACTIVE MATERIALS USED EACH DAY (Appendix K).

Attach to all radioactive waste containers, a Radioactive Waste Pick-up Receipt (NIH 88-35) labeled with the type of isotope, the approximate amount of radioactivity present in mCi, the date and the initials of the laboratorian.

A daily survey is required whenever radioactive materials in unsealed form are manipulated. A survey using an instrument such as a Geiger-Mueller counter is acceptable as long as it is sensitive enough to detect the nuclide used. The use of the nuclide I-125 requires a survey instrument equipped with a low energy sodium iodide crystal. For low energy beta emitters such as H-3, C-14, S-35 or P-33, contamination surveys shall be conducted using swipes (or smear wipes), which are counted using a liquid scintillation counter.

Any area found to be contaminated must be decontaminated immediately. If an item of equipment is contaminated, but has been dedicated for continued use with radioactive materials where re-contamination is likely, it shall be labeled as contaminated and dedicated for use with radioactive material.

An area where radioactive materials are used or stored shall be appropriately posted with a visible "Caution Radioactive Materials" sign. The sign must indicate the Authorized User responsible for the room, along with telephone numbers for business and after hours contact. Once each month, a complete contamination survey of each posted area must be completed using swipes. At least ten probable places of contamination must be surveyed and the swipes counted by appropriate techniques. Any radioactive contaminated areas found during the monthly survey must be decontaminated. This survey, including a diagram of the lab showing locations of major equipment and swipe areas, is required to be documented on form NIH 88-12, "Monthly Laboratory Contamination Survey." After completion, the original copy of the survey must be submitted to RSB. Additionally, one copy must be filed in your Radiation Safety Records book for the lab. These surveys shall be retained for three years in your records book for inspection by RSB personnel or NRC inspectors.

2.3.3 Waste Storage Removal

Radioactive waste material will be stored in containers behind Plexiglas shields until removed from the MCL. Waste materials will be tagged with Radioactive tape and labeled with the type of isotope, the approximate amount of radioactivity present (in total mCi), the infectious agent, the date and the initials of the person who generated the waste. Solid waste will be stored in autoclave bags in a designated radiation storage area and will be removed from the MCL by autoclaving. Due to the potential for contamination by autoclaving of radioactive liquids, this type of waste will be decontaminated by the addition of a suitable decontamination solution to inactivate any potentially infectious agents and removed when the laboratory is decontaminated for annual maintenance. All radioactive waste with a half-life of <100 days must be separated from waste with a half-life of >100 days.

In the event of a radioactive spill that precludes adequate cleanup, the event will be reported to the Facility Manager and/or the MCL Occupational Safety and Health Specialist who will notify the RSB Health Physicist.

2.4 EXPERIMENTAL ANIMALS

2.4.1 General

Animal research shall follow the guidelines established by the NIH Animal Research Advisory Committee (ARAC), the American Association for Laboratory Animal Science (AALAS), and the American Association for Accreditation of Laboratory Animal Care (AAALAC), the publication entitled *Guide for the Care and Use of Laboratory Animals, and the PHS.*

1. The housing, care, and handling of animals must conform to the current guidelines specified in the Institute of Laboratory Animal Resources (ILAR) publication entitled Guide for the Care and Use of Laboratory Animals.
2. All protocols involving the use of animals shall be approved by the appropriate NIH ICD Animal Care and Use Committee and the Chief, OSHB.
3. The use of sharp instruments in the MCL should be kept to a minimum. The use of glass is prohibited unless specifically approved by the Facility Manager and/or Occupational Safety and Health Specialist. Needle-locking (Luer-Lok) hypodermic syringes shall be used when syringes are necessary.
4. Doors to the laboratory/animal rooms must be kept closed at all times.

5. Only trained personnel are permitted to handle the experimental animals. The handling of infected animals, alive or euthanized, and secretions/excretions from such animals should be done carefully to minimize contamination of the laboratory area and damage to the positive pressure suit.
6. Squeeze-back cages will be used to physically control and house all nonhuman primates. Nonhuman primates must be anesthetized during procedures that require close contact or handling by personnel in the MCL.
7. All animals must be euthanized and then autoclaved (121.5 °C X 65 min) prior to removal from the MCL. Autoclaved bags shall be placed in MPW boxes and disposed of in accordance with NIH policy and procedures. See section 2.4.5.
8. All animal tissues for histopathology must be decontaminated prior to removal from the MCL. For this purpose, tissues will be placed in an ample volume of an appropriate fixative in a screw cap bottle for a minimum of 72 hours, transferred to a new bottle containing fresh fixative, and then removed from the MCL via the dunk tank in the equipment room to the Facility Core Center. The appropriate fixative shall be determined prior to initiation of a research project.
9. All blood and serum specimens removed from the MCL for serologic testing must be in screw cap vials. The exterior of the vials shall be (i) decontaminated, (ii) double-bagged using heat-sealed plastic bags, and (iii) removed from the MCL through the dunk tank. Virus in the samples should be inactivated by gamma-irradiation from a 60Co source (5 X 106 rads) before the plastic bags are opened. Animal sera should be exposed to 2 X 106 rads.

2.4.2 Inoculation of Animals with Infectious Material

1. Inoculation of experimental animals will be done only in a BSC. All animals must be anesthetized for inoculation with an infectious agent to avoid accidental injury, to the human handler(s).
2. Syringes and hypodermic needles shall be discarded into a sharps container immediately after use. Needles shall not be recapped after use. When the sharps containers are three fourths full, they shall be placed in autoclave pans and autoclaved for removal from the MCL.

2.4.3 Collection of Biological Samples from Live Animals

1. The collection of biological materials (including blood samples and bodily excretions or secretions) from live animals should be done in the BSC in the animal/laboratory room. If the animal is too large to be restrained in the BSC, the protocol requesting this exemption, shall be submitted to the PRC prior to the initiation of the research project.
2. Venipuncture sites shall be swabbed with an appropriate disinfectant.
3. Syringes and needles shall be discarded into a sharps container immediately after use. Needles shall not be recapped after use.

2.4.4 Housing Infected Animals

1. Only cages and water bottles approved by OSHB will be used for animals in the animal/laboratory suites.
2. Cages housing infected animals must be labeled to indicate the name of the infectious agent(s) in use, and the name and phone number of the person responsible for the care of the animals.
3. Animals in cages with contact bedding should be transferred to clean cages at least once each week unless otherwise directed. Care should be exercised to minimize aerosols from soiled cage bedding and other refuse. All used cages and refuse from the animal rooms must be autoclaved (121.5 °C X 90 min) before removal from the MCL.

2.4.5 Handling Animals

1. Care shall be exercised handling animals and their housing units to minimize the aerosolization of infectious materials.
2. Whenever feasible, disposable surgical or medical gloves shall be worn over the outer gloves on the positive pressure suit when handling infected or potentially contaminated animals.
3. While performing surgical procedures on infected animals, a disposable gown shall be worn over the positive pressure suit and disposable gloves over the suit gloves. The BSC surface should be washed down with an appropriate disinfectant upon completion of the procedure(s). Used instruments shall be placed in a discard pan or tray containing an appropriate decontaminating solution for the prescribed period of time, cleaned and sterilized.
4. Forceps or gloved hands shall be used to remove dead animals from cages. Forceps shall be stored in an appropriate disinfectant solution and gloves shall be washed with disinfectant after handling dead animals.
5. Dead animals shall be placed in closed leak-proof, double bags before transport to an autoclave, refrigerator, or freezer. Each container shall be identified with the date, investigator's name, and the infectious agent.
6. All animals must be euthanized and then autoclaved (121.5 ˚F X 90 min) prior to removal from the laboratory. Euthanized animals shall be stored in a refrigerator or freezer if the autoclave is not available for immediate use. Euthanized animals shall be autoclaved in an open bag containing water. Autoclaved bags shall be placed in MPW boxes, labeled and properly disposed of in accordance with NIH policy and procedures.
7. An animal that escapes during handling shall be considered potentially contaminated with the infectious agent(s) in use the MCL. Consequently, such animals shall be euthanized unless the responsible investigator directs otherwise.

2.4.6 Necropsy

1. All necropsies of animals will be done in the BSC, or on a downdraft necropsy table within the MCL.
2. Disposable gloves and disposable gowns must be worn over the positive pressure suit when performing necropsies on infected or potentially contaminated animals.
3. Care should be exercised when performing a necropsy to prevent damage to the protective suit by sharp bone fragments or instruments.
4. Upon completion of a necropsy, all animal tissues not saved for histopathology shall be placed in appropriately labeled, leak-proof bags, and autoclaved. Autoclaved bags should be removed from the autoclave, double bagged and placed in MPW boxes. All boxes should be sealed and labeled by the laboratorian. Consult the MCL Occupational Safety and Health Specialist for information on proper disposal of the MPW boxes. All instruments should be placed in the appropriate decontaminent for the prescribed period, scrubbed, and autoclaved. The BSC or downdraft table surface shall be cleaned with the appropriate decontaminanting solution.
5. The disposable gown and gloves worn when performing a necropsy shall be placed into an autoclave bag, autoclaved, and disposed of in a MPW box in accordance with NIH policy and procedures.

2.5 REGISTRATION, STORAGE, AND TRANSPORT OF INFECTIOUS AGENTS

2.5.1 Registration of Infectious Agents

All BSL-2, 3, and 4 agents as defined in "Biosafety in Microbiological and Biomedical Laboratories" (HHS Publication No. [CDC] 93-8395) will be registered and approved for use in accordance with NIH guidelines.

A Registration of Materials (Potentially) Infectious for Humans (HPRD) Appendix R, must be approved by the NIH IBC and OSHB before studies with the agent(s) are initiated.

The Chief, OSHB, must approve all infectious agents or materials brought to the NIH. Certain agents require that PHS and United States Department of Agriculture (USDA) permits for the transport of the agent be obtained prior to shipment of the material from point of origin to the NIH. If the agent has not been previously handled at the NIH, OSHB shall be notified of the nature of the agent and its intended use.

2.5.2 Storage of Infectious Agents

1. Infectious or toxic materials shall be stored only in refrigerators, incubators, or freezers which are marked with the universal biohazard symbol.
2. Transportation of all infectious or toxic materials within the MCL shall be placed within a secondary, unbreakable container.
3. All infectious or toxic materials stored in refrigerators or freezers must be properly labeled and stored in containers capable of withstanding the thermal shock of freezing and thawing. GLASS IS NOT ALLOWED IN THE MCL WITHOUT EXPRESS APPROVAL OF THE FACILITY MANAGER. Each container should be labeled with the identity of the infectious agent, the date of the preparation, the name of the responsible laboratorian and a reference number which links the material to the more inclusive information contained in the inventory databases.
4. When work is completed and prior to exiting the MCL, all infectious cultures or toxins will be removed from work benches and cabinets and stored in a designated refrigerator, incubator or freezer. Material to be discarded will be placed in a sealable container, placed in a discard pan containing a decontaminant, placed on a cart and transported to the autoclave.
5. Labware containing infectious liquids must be stored and transported in a leak proof container which has sufficient capacity to contain the liquid (in the event of breakage of the labware).

2.5.3 Removal and Transport of Infectious Materials

Infectious Agents

1. Containers of infectious or toxic substances for transport from the MCL will be placed in a larger, unbreakable container having solid sides, bottom, and a leak proof cover. The surface of the carrier shall be decontaminated by passage through the dunk tank.
2. Infectious, toxic, radioactive or recombinant DNA materials shall be shipped off-campus in accordance with NIH/CDC Safety Guidelines and Department of Transportation (DOT) shipping regulations. The Chief, OSHB, or his/her designee will approve all requests for shipments of infectious materials. When the transfer permit is approved, the shipment will be handled by the NIH Shipping Office. If the infectious agent is exotic in origin, the recipient of the material must have a PHS (and possibly USDA, and/or United States Fish and Wildlife Service [USFWS]) permit to ship infectious agents. Permit labels shall be attached to the package (UN Class 6.2) with the appropriate "BIOHAZARD INFECTIOUS MATERIAL" labels.
3. The Chief, OSHB, will approve all shipments of BL-4 agents.
4. The shipment of any material containing radioactivity shall be handled by RSB: (i) An appointment must be made with the Materials Control Unit (6-3277), (ii) 24 hour advance notice is required by RSB.

Tissues for Histopathology

5. Prior to removal from the laboratory, tissues should be processed as follows:
6. The tissue should be sectioned into small pieces that are easily fixed. Generally, sections should be less than 1 cm. cubes.

7. The material should be placed in at least 10 X volume of an appropriate fixative. For most purposes, 2% glutaraldehyde can be used for fixation of tissues intended for electron microscopy.
8. The fixed material should be changed to fresh fixative in a new screw cap container 3 days after initial fixation. The container of fixed material may be surface decontaminated and removed from the MCL area by submersion in the dunk tank in the equipment room. The fixed material shall be placed in a leak proof, unbreakable container for transport from Building 41A.

2.6 WASTE MANAGEMENT IN THE MCL

All infectious or toxic materials, contaminated reusable labware and contaminated waste will be autoclaved prior to washing or disposal by the laboratorian. Contaminated materials will be placed directly into an autoclave or held in a covered container for subsequent autoclaving. Water should be added to all containers to be autoclaved. After autoclaving, package all disposable items in MPW boxes in accordance with NIH policy and procedures.

Radioactive waste: Contact the Health Physicist, RSB (6-5774), to arrange pick up of materials (see Radiation Safety Manual). Arrangement for autoclaving and pick up is the responsibility of the individual who has generated the specific radioactive waste.

Items will be segregated before autoclaving as follows:

1. Syringes, needles, and other sharp objects: place in authorized sharp containers with no re-capped needles.
2. Disposable soft materials: paper and plastic, plastic wrapping from pipettes and plastic pipettes shall be placed in autoclave bags. Autoclave bags shall be placed in a leak proof pan.
3. Other disposable materials: vessels, tubes, pipettes, and metal shall be placed in an autoclavable container/or pan.
4. Reusable labware: place in autoclavable containers. Autoclave out of the MCL, transport to the appropriate Institute for washing.
5. Animal cages: animal cages are to be placed in the autoclave with as little disturbance to bedding as possible.

2.7 MAINTENANCE SUPPORT ACTIVITIES

2.7.1 Shutdown of the MCL

The MCL is decontaminated annually or at the end of each research project (whichever occurs first), and will be used to service all scientific and communication equipment of the MCL. All investigators will be responsible for completing the AMCL Checkout Procedure (Appendix L) at the end of the research project. This shutdown is coordinated with DES, and will be used to inspect all aspects of the integrity and safety features of Building 41A. The protocol and list of responsibilities for the gas decontamination of Building 41A is in Appendix M. Refer to Section 2.8 for staff responsibilities.

2.7.2 Decontamination Airlock Area

In the event that equipment must be removed from the MCL prior to the annual Facility decontamination, the item is cleaned of all chemical and biological material, and moved to the Decontamination Airlock area (see section 3.3.2) to be decontaminated prior to removal from the Facility. See Appendix N for VHP procedures.

2.8 MAINTENANCE DUTIES OF STAFF

2.8.1 Facility Manager

The Facility Manager shall coordinate maintenance duties with DES and the MCL Occupational Safety and Health Specialist. In the absence of the MCL Occupational Safety and Health Specialist, other OSHB staff members will be responsible for these duties. Assignment of these alternates will be made by the Chief, Safety Operations Section, OSHB.

The Occupational Safety and Health Specialist shall be responsible for the following:

1. Autoclave Quality Assurance.
2. Ensure that the decontaminant in the Decontamination Shower tank and dunk tank are maintained at the appropriate level.
3. The coordination of the removal of the decontaminated waste and material from the autoclave and dunk tank.
4. Maintain an adequate supply of laboratory disposable jump suits, clean towels, soap, and shampoo in the dressing room.
5. Place used laundry in bags for pick-up by Housekeeping.
6. Ensure that suit rooms are supplied with caps, inner gloves, heavy-duty gloves to be taped to the suits, tape, scissors, and 70% alcohol.
7. Perform the critical systems check daily.
8. Maintain entry and exit records on the computer database, monitor the closed circuit TV system and issue the proximity card keys.

2.8.2 All MCL Laboratorians

Before entering the MCL:

Ensure that the Critical Systems checklist (Appendix G) has been completed. This checklist shall be on file in the Facility Core Center. A notice will be posted in the outer change room upon completion of the Critical Systems checklist. This inspection is completed daily by the Facility Manager or the MCL Occupational Safety and Health Specialist.

Inside the MCL:

1. The first person who enters the MCL each day must complete the Interior Checklist (Appendix O) to assess any malfunctions of the systems.
2. Keep the MCL change room and suit room clean and orderly.
3. Place all trash in autoclave bags, add water, and autoclave. On the clean side of the autoclave, at the end of the autoclave cycle place all autoclave bags in MPW boxes. All MPW boxes shall be disposed of in accordance with NIH policy and procedures.
4. After each use of the BSC, pour one to two inches of the appropriate decontaminating solution into the bottom of each discard pan used in the BSC and autoclave discard pan.
5. Clean BSC work surface after each use with appropriate decontaminating solution. Place clean discard pans in the BSC.
6. Autoclave all waste animal food, animal waste pans and cages before removal from the MCL. (See 3 above)

DISPOSAL Of EUTHANIZED ANIMALS AND ANIMAL TISSUES IS THE RESPONSIBILITY OF THE INVESTIGATOR. Place animals/tissues in autoclave bags, add water, and autoclave.

After exiting the MCL:

REMOVE THE BAGS WITH THE EUTHANIZED ANIMALS AND ANIMAL TISSUES FROM THE AUTOCLAVE, DOUBLE BAG AND PLACE IN MPW BOXES. PLEASE CONSULT THE MCL OCCUPATIONAL SAFETY AND HEALTH SPECIALIST TO COORDINATE APPROPRIATE PICKUP OF MPW BOXES IN ACCORDANCE WITH NIH POLICY AND PROCEDURES.

3.0 BUILDING SYSTEMS

3.1 BUILDING OVERVIEW

Building 41A houses the MCL which is designed as a BL-4 laboratory. The building contains a Facility Core Center, mechanical areas on both east and west sides of the building with air handling equipment housed in the area above the MCL. Entrance into the building is through proximity card accessed doors.

The MCL consists of three laboratory/animal suites, one equipment room, autoclave staging area, decontamination airlock, VHP control room and a small storage closet. Building 41A has many special features.

1. Filtration of supply air is through high-efficiency particulate air (HEPA) filters. Exhaust air is filtered through two HEPA filters in series before being discharged.
2. Laboratorians wear impermeable, positive pressure, supplied breathing air suits while working in the MCL. The air supply is regulated for breathing and suit cooling.
3. All liquid effluent is decontaminated. This Liquid Effluent Treatment System involves steam sterilization, cooling and neutralization to assure proper pH and temperature of effluent is achieved before release to the sanitary sewer.
4. The entry corridor and mechanical areas are "clean", however, for security purposes, access is proximity card controlled.
5. Two double door autoclaves with interlock mechanisms which are accessible from the "clean" corridor as well as the MCL. Solid waste is removed from the MCL only after autoclaving.
6. Access to the laboratory suites through a clean change room. This area contains lockers for storage of personal clothing, supplies of disposable jump suits and gloves to be worn under positive pressure suits, HEPA filters for air lines, clean towels, soap and shampoo for the personal shower and laundry bags for disposal of soiled linen. A toilet and personal shower are located in the inner change area.
7. A suit room contains the positive pressure suits, heavy duty gloves and supplies for attaching gloves to suits.
8. The decontamination shower has interlocking doors. The boot area is located just beyond the decontamination shower in the MCL main corridor.
9. Each laboratory/animal suite contains a BSC.
10. A supply closet with limited storage capabilities for operating supplies.
11. A manually operated emergency shower and emergency breakout panel located at the south end of the main corridor of the MCL.
12. A Decontamination Airlock is located at the north end of the MCL main corridor for VHP decontamination.

3.2 SECURITY

3.2.1 Monitoring of Facility

The MCL security is provided by 24 hour camera surveillance and the NIH police who patrol the NIH campus.

3.2.2 Authorization of Personnel for Entry

Access to Building 41A and the MCL is restricted to those personnel who must enter for program or support needs. Such employees will (i) be briefed on the potential hazards of the BL-4 agents handled in the laboratory, (ii) be familiar with the standard and emergency procedures described in the Safety and Operations Manual, Building 41A, and (iii) participate in the required MCL training. New staff members shall work in the MCL only after receiving authorization from the Chief, OSHB, or the Facility Manager. A minimum two-week training period shall be preceded by an orientation to the MCL presented by OSHB.

Persons who are not an employee of the NIH but who are Apeer scientists/collaborators, may qualify for entry or work in the MCL but only with approval of the NIH IBC, PRC, and Chief, OSHB. Non-employee visitors will be briefed on the potential hazards of the laboratory by the Facility Manager and/or the Occupational Safety and Health Specialist. These visitors will be familiar with the procedures described in the Safety and Operations Manual, Building 41A and attend the required MCL training.

Authorized service personnel may enter the Facility Core Center corridor or the mechanical areas for routine monitoring and service during duty and non-duty hours. Persons other than MCL staff, will not enter the MCL when the facility is operational and viable materials are present unless approved by the Chief, OSHB, or the Facility Manager and/or the Occupational Safety and Health Specialist.

3.2.3 Facility Utilization

Use of the facility shall be limited to programs approved by the NIH IBC, PRC and the Chief, OSHB. No changes and/or additions to approved programs and projects can be made without written approval of the foregoing.

3.3 SYSTEMS

3.3.1 Alarms

Visual strobes of 70,000 candle power are located throughout the MCL to indicate a fire emergency or failure of the HVAC and breathing air systems. Telephones and fax machines are available for normal and emergency use. Alarm indicators are located in the Facility Core Center and indicate the following: (i) decontamination tank level; (ii) air balance inconsistency; (iii) unauthorized entry and (iv) improper use of interlocked doors. Mechanical system alarms are automatically relayed to the Building 41A engineer, (East Mechanical Office in Building 41A), and South Maintenance Engineering in Building 37.

3.3.2 Decontamination Airlock

The Decontamination Airlock serves as an airlock between the main corridor of the MCL and the exterior of Building 41A. This space may be used to surface decontaminate large pieces of equipment using the VHP unit before removal from the MCL (Appendix N). If necessary, formaldehyde can be used as a decontamination agent. The doors of this space are interlocked and are only opened with a manual key lock from within the MCL.

3.3.3 Autoclaves

Two double-door autoclaves, one large and one small, are in the MCL. Each is interlocked so that it cannot be opened to the clean corridor of Building 41A until a sterilization cycle has been completed. Standard operating procedures are found in Appendix P. All solid waste, discard pans, animal cages, animal waste, bedding and feeding apparatus are sterilized by autoclaving before removal from the MCL. The automatic timing system on the autoclaves places the autoclaves in a stand-by mode. Between the hours of 6:00 am - 7:00 pm, Monday - Friday, the autoclaves are in an operational mode. Autoclaves are maintained by a maintenance contractor. Notify the MCL Occupational Safety and Health Specialist immediately if a problem occurs.

3.3.4 Biological Safety Cabinets

The MCL contains three Class II, Type A laminar flow BSC, one in each laboratory/animal suite. The cabinets are certified annually as part of the facility maintenance.

3.3.5 Breathing Air (Suit Air)

Breathing air is supplied from specially designed compressors through constant temperature air dryers. Mechanical refrigeration is used to remove moisture from compressed air while providing a constant outlet air temperature. The dryers are designed to deliver the air at constant temperatures. The facility is equipped with redundant air compressors to minimize disruption of service.

In the event of the failure of both compressors, emergency bottled breathing air is supplied through a duplex manifold which is designed for service in locations where a constant and adequate supply of air is needed. The manifold is designed to changeover from one bottle to another automatically. It is recommended that in the event of failure of the breathing air compressors, MCL personnel shall store project work and exit the MCL. Strobes will indicate such an emergency (see section 3.3.1) throughout the MCL. The mechanical system alarm panel will indicate an alarm state in (i) the Facility Core Center, (ii) East Maintenance Office, Building 41A and (iii) South Maintenance Unit Office, Building 37.

3.3.6 Laboratory Gases

The laboratory compressed gas manifold is designed to provide an uninterrupted supply of gas to the MCL piping system. The manifold provides for automatic changeover from the depleted bottle to a secondary bottle with no fluctuation in delivery pressure. Pressure gauges on the manifold indicate the system status and alert staff about the need to replace any depleted cylinders.

3.3.7 Communications

Communications between the MCL and the Facility Core Center or staff may be accomplished in four ways:

1. intercom
2. telephone
3. fax
4. 2-way headphone systems worn inside of the personal protective suit.

The intercom system operates between the Facility Core Center, dressing room and the MCL. Telephone and fax can be accessed to anyone outside the MCL. See Appendix C for a list of telephone numbers. They are primarily used for communication between personnel inside the MCL and the OSHB staff. The fax is used to send data and other written communication out of the MCL. Books and papers cannot be removed from the MCL.

3.3.8 Decontamination Shower

The facility has a decontamination shower, which also serves as an air lock between the MCL and the "clean" area. All personnel enter and exit the MCL through the decontamination shower/air lock (Appendix I).

The decontamination shower is equipped with interlocking doors having inflatable gaskets. Inflation of the gasket is controlled with the door handle; depressing the handle deflates the gasket. It is essential that personnel ensure complete closure of the door after passage, to allow the door gasket to fully deploy and maintain containment.

When exiting the MCL, enter the decontamination shower next to the boot area. Initiate automatic cycle by pressing the green push-button labeled “START” located on the upper left side of the MCL shower door. When the automatic cycle is completed, press the door handle down, allow the gasket to deflate and exit the shower.

Gravity feed operation of the shower in the event of a power failure or accident is performed by using the manual pull handle suspended from the ceiling of the shower. Pull the manual handle to release decontaminating solution; to stop the manual shower, release handle.

3.3.9 Emergency Showers

An emergency shower, which operates identically to the manual shower in the decontamination shower, is located at the south end of the main corridor of the MCL. The pull chain is located in front of the break out panel. This shower and break out panel are to be used only in life threatening situations.

3.3.10 Ventilation

The airflow within the MCL is of critical importance to the containment function of the laboratory. Outside air enters through HEPA filters and airflow is of a directional nature, proceeding from areas of higher pressure (clean side) into areas of lower pressure (MCL). All air is double HEPA filtered before being exhausted to the outside.

3.3.11 Liquid Effluent Treatment System

All liquid waste is discharged through a closed system into decontamination tanks located in the sub-basement of Building 41. Each tank is steam processed and subsequently, partially cooled. The contents are then transferred to other tanks for pH neutralization and further cooling. This processed waste effluent is then allowed to enter the sanitary sewer system. (See section 5.6) The operation of the Liquid Effluent Treatment System is the responsibility of the Division of Safety.

3.4 LABORATORY EQUIPMENT OPERATION

3.4.1 Carbon Dioxide Incubators

CO_2 incubators shall be moved into the MCL when the program requires them.

The investigators shall maintain this equipment.

3.4.2 Centrifuges

An ultracentrifuge and/or a centrifuge required for a current project shall be located in the equipment room. Maintenance will be performed as needed by MCL staff or by an authorized service representative during the annual shutdown of the MCL. Tabletop centrifuges are located in the laboratory/animal suites.

3.4.3 Water Baths

Water baths shall be located in the laboratory/animal suite as needed. Maintenance is performed during the annual shutdown and as needed. All water baths are checked according to standard laboratory practices. Water baths shall (i) not have mercury thermometers; (ii) have automatic shutoffs for low water level and (iii) over temperature controls.

3.4.4 Microscopes

Any microscopes required for the research program shall be located in the laboratory/animal suite of the MCL. Microscopes shall be serviced annually during shutdown. Maintenance is performed as needed.

3.4.5 -20°C and -80°C Mechanical Freezers

Freezers shall be located in the MCL equipment room or as required by the research program. Repairs and maintenance are performed as needed and during annual shutdown.

3.4.6 Refrigerators

Laboratory refrigerators and a refrigerator for storage of animal food shall be located in the equipment room of the MCL.

3.4.7 Scintillation Counter

If radioactive material is used in the MCL, the radioactive material shall be contained in scintillation vials and removed from the MCL through the dunk tank. Access to a liquid scintillation counter will be required by RSB.

3.5 ANIMAL SUITE

3.5.1 Housing

Animals are housed in appropriate cages recommended by the "Guide for the Care and Use of Laboratory Animals" (U.S. Department of Health and Human Services published 1996) unless other arrangements have been approved by the ARAC.

3.5.2 Sanitation

Cages are changed according to species. All cages, pans and bedding are autoclaved out of the suite. The Institute using the MCL for a research project shall be responsible for changing and washing the cages. The animal suite is cleaned by the laboratory staff responsible for the animals.

3.5.3 Food

Food for all animals is obtained by the animal care staff, and moved into the MCL via the large autoclave. The food shall be stored in closed containers. All uneaten or spoiled food is autoclaved out of the laboratory before being discarded.

3.5.4 Records

Animal records are maintained within the MCL until experiments are completed. Records are attached to the cage; with the exception of non-human primate records which are kept in a notebook. The records may be transferred only by facsimile at any time.

3.5.5 Pest Control

An integrated pest management program (IPM) is in place for Building 41A (Appendix Q). A synopsis of the integrated pest management plan follows.

1. IPM is an approach to controlling pests that minimizes reliance on the use of pesticides and emphasizes management of the environment (i.e., personnel procedures and facility conditions) to prevent pests from becoming a problem.
2. The rigorous sanitation and maintenance requirements associated with operating this facility should preclude the establishment of pest "infestations" within the containment area. The most likely source of pest problems will be the incidental ingress of pests through doorways, and the introduction of pests with animal feed and goods/supplies brought from home or laboratories.
3. Pest management surveys inside the containment area will occur upon request of the Facility Manager, in order to diagnose and resolve specific pest issues. The exterior of the building, the mechanical rooms, the locker room and the Facility Core Center will be monitored and observations recorded in a logbook kept in the Facility Core Center. One Pest Management Unit staff member, and a back up, will be assigned to perform this task.

4. The primary pest control tactics used in Building 41A will be non-chemical, i.e., traps, exclusion, removal/disposal. If the use of a pesticide is necessary, only baits and solid formulations of pesticides will be used. This will eliminate the potential for drift and volatilization of petroleum distillates and solvents associated with the use of some liquid and aerosol formulations of pesticides.
5. An IPM logbook will be compiled and maintained by pest management personnel. This logbook will contain MSDSs and labels for all pest management products that may be used in and around Building 41A. In addition, the logbook will contain protocols and procedures for performing routine and emergency pest management services and reporting pest activity, IPM reports and recommendations, and pest data.

3.6 ANNUAL INSPECTION AND PREVENTIVE MAINTENANCE

An annual inspection of the MCL is carried out by OSHB and coordinated with the Division of Engineering Services to inspect all aspects of the integrity and safety features of Building 41A. Maintenance of the scientific and communications equipment is done during the annual shut down.

4.0 EMERGENCY PREPAREDNESS PLAN

4.1 INTRODUCTION

This plan establishes the procedures to follow in the event of a fire, medical emergency or bomb threat at the NIH Maximum Containment Laboratory (MCL), Building 41A. The priority consideration in the event of an emergency is the protection of the health and safety of personnel working in the MCL.

Safety procedures described in this plan shall apply to all MCL activities and all program and support personnel. Modifications to these procedures or to the facility shall not be made without written approval of the NIH Institutional Biosafety Committee (IBC), Policy Review Committee (PRC) and the Chief, Occupational Safety and Health Branch (OSHB).

4.2 DEFINITIONS

1. Biosafety Level (BL). A combination of laboratory facilities, safety equipment and microbiological procedures used in handling etiologic agents, encompassing four levels of potential hazard with BL-1 providing the least risk and BL-4 the highest. This combination is appropriate to the potential hazard of the etiologic agent in question and is designed to protect the worker, environment and the community.
2. *CHEMTURION* Suit. A totally encapsulating, positive pressure biological/chemical protective suit constructed of 20mil chlorinated polyethylene. Breathing air is supplied to the suit by an umbilical hose.
3. Decontamination. The physical or chemical process by which an object, area, or person contaminated with a harmful or potentially harmful etiologic agent, is made safe for handling or use.
4. Emergency. An event in the MCL which may involve: 1) exposure or injury of personnel, 2) compromise of biological and/or physical barriers or 3) major equipment failure which could compromise safe operations in the facility. Refers to all situations (fire, medical, etc.) in which a rapid response is necessary to limit injuries to MCL personnel and/or maintain the operational integrity of the facility.
5. Fire and Emergency Response Section. The NIH Fire Department is the primary responder to all fire, rescue, technological and medical emergencies on the Bethesda campus.
6. Incident Commander (IC). The senior emergency response professional (fire or police department representative) on the scene, who is in charge of the incident scene and responsible for all decisions pertaining to the management of the emergency situation.

4.3 RESPONSE TO FIRES

In the event of a fire in the mechanical spaces or office area, efforts to extinguish the fire by MCL personnel should be attempted only after the fire department has been notified, and if the fire is limited in size and egress from the area will not be compromised. Do not attempt to fight a fire in an animal/laboratory room while wearing a *CHEMTURION* suit.

Open flames are not to be used in the MCL, unless it involves repairs to the laboratory and a hazardous work permit has been obtained and the proper safeguards are in place. There are fire extinguishers and an automatic, quick response sprinkler system located throughout the Facility Core Center, animal/laboratory rooms and mechanical spaces of Building 41A. The MCL has a primary exit, the personal decontamination shower room and a secondary exit, the emergency breakout panel at the south end of the marshaling corridor. There is a manual fire alarm pull station located near the primary and the secondary exit and near the entrance door to the Facility Core Center. All program and support personnel should familiarize themselves with the location of all exits, fire extinguishers and manual fire alarm pull stations. Section 5.2 is a Floor plan of Building 41A showing the locations of these fire safety devices.

If you are in the office or change room area and see smoke or flames:

1. Notify everyone in the MCL of the fire by pulling the nearest fire alarm manual pull station and phoning 911 - tell the NIH Fire Department your location, name and the nature of the emergency.
2. Don gloves and go to the decontamination shower area and assist individuals in removing their *CHEMTURION* suit. In a life-threatening emergency, immediate personal safety overrides maintenance of containment. Evacuation takes priority and a decontamination shower is not required.
3. Exit and move away from the building. The facility manager or his/her designee will report to the Fire Department IC and provide details of the emergency including whether or not all MCL staff have safely evacuated the building, the nature of the emergency and the biological agents in use.
4. In the event that additional decontamination steps may need to be taken, all MCL staff must remain in the same area. Potentially contaminated MCL personnel shall not make physical contact with other individuals.

If you are in an animal/laboratory room and can safely do so:

1. Turn off all electrical appliances.
2. Secure all infectious materials in a refrigerator, freezer, or incubator.
3. Close all animal cages and leave the cages in place in the room.
4. Close the laboratory door as you leave the room.
5. Proceed to the primary exit and leave the facility through the decontamination shower. In a life threatening emergency, a decontamination shower is not required.

If the primary exit is blocked, proceed to the emergency breakout panel at the south end of the marshaling corridor and leave the MCL.

1. Remove the *CHEMTURION* suit once you are safely outside the MCL. Minimize any contact with the outside of the suit or gloves and place the suit inside-out on the ground.
2. Move away from the building to the designated marshaling area and report to the Facility Manager or his/her designee. Potentially contaminated personnel will avoid any contact with other individuals. Additional decontamination steps may need to be taken.

If you are in either the east or west mechanical room and see smoke or flames:

1. Activate the alarm by pulling the nearest fire alarm manual pull station and dial 911 - tell the NIH Fire Department your location, name and the nature of the emergency.

2. Use a fire extinguisher only when the fire is limited in size and egress from the room will not be compromised.
3. Immediately exit the facility and report to the designated marshaling area.

4.4 RESPONSE TO MEDICAL EMERGENCIES

This section outlines responsibilities and procedures for response to the various types of medical emergencies which may occur within the Maximum Containment Laboratory.

4.4.1 Potential Exposure To Biological Agents

Any potential exposure shall be reported to the Occupational Medical Service (OMS) and to the Facility Manager (or the MCL Occupational Safety and Health Specialist) and the Chief, OSHB immediately. An evaluation will be made as to the actual risk and the course of action to be taken.

If a significant risk is judged to have occurred, then the OMS, will be responsible, in consultation with appropriate medical specialists and the OSHB, in decisions regarding the management of the exposure situation. Management may range from no action, other than correction of the conditions that led to the situation, in the case of negligible risk; to close monitoring of the person for fever and/or other symptoms; to isolation and possibly treatment with prophylactic drugs or other modalities.

4.4.2 Potential Skin Or Aerosol Exposure To Biological Agents

Any breach in the integrity of the *CHEMTURION* suit will be managed as described in section 2.2.5 (Suit Malfunction) of the *Maximum Containment Laboratory Safety and* Operations Manual. After the laboratorian exits the MCL, the exposure will be reported to the OMS for medical monitoring and counseling. Also, the Facility Manager (or the Occupational Safety and Health Specialist) and the Chief, OSHB will be notified. If the exposure is judged to be of very low risk, the employee will be instructed to be alert for any possible signs of illness. If the exposure is thought to be of potential significance, management will be discussed between the OMS, appropriate medical specialists and the Chief, OSHB. The IBC and PRC will be informed if the risk level is judged to be high.

4.4.3 Potential Percutaneous Exposure To Biological Agents

1. The wound site will be treated with a disinfectant, available at each sink in the MCL, for a minimum contact time of ten minutes.
2. The person will exit the MCL immediately, following established decontamination shower procedures.
3. Additional application of disinfectant and thorough washing of the affected area for ten minutes with soap and water will be conducted in the change room lavatory.

These events will be reported to the MCL Facility Manager (or the Occupational Safety and Health Specialist), the Chief, OSHB and the OMS as soon as feasible. Routine first-aid and an accident report for the cut or needle injury will occur through the OMS according to NIH policy. The post exposure management of the infectious hazard will be discussed between the OMS, appropriate medical specialists and the Chief, OSHB; if the risk level is judged to be high, the IBC and PRC will be notified. A NIH Notice of Traumatic Injury Form must be completed.

4.4.4 Health Emergencies Within the MCL

Effective treatment of known life-threatening situations must take precedence over the threat of the MCL microbial environment. Because the major risk to emergency medical personnel is through aerosols, their entry into the MCL suites should generally not be permitted. If there has not been major contamination of the affected worker, decontamination of the suit exterior in an emergency can be less thorough than in

ordinary circumstances, particularly given the Blood borne pathogen protection that is exercised by professional Emergency Medical Technicians (EMTs). The telephone should be used to notify persons outside the MCL to mobilize assistance. All of the following procedures must be done promptly to ensure rapid, safe patient transport to definitive medical care.

1. Call for help: Dial 911.
2. Give your location and the type of medical emergency.
3. Provide basic first aid if possible.
4. Emergency response staff will enter the containment area and will be responsible for extracting and decontaminating a patient(s) with serious injuries or other life-threatening conditions. Special considerations include:

4.4.5 Handling of Ambulatory Patients

In a situation where the patient can walk with assistance, without compromising their well-being, the patient may take a routine decontamination shower and exit in the normal manner.

1. Protective clothing: It may be necessary to remove the patient's protective *CHEMTURION* suit while still in the MCL. The positive pressure suit surface is sprayed with the disinfectant appropriate for the biological agent(s) in use, prior to removal of the suit. The suit may be doffed after it has been sprayed with the disinfectant. The patient may then be treated for injuries or illness by the emergency response personnel.
2. Decontamination of the patient: An evaluation must be made by the Facility Manager to ensure that the patient was not exposed to a BL-3 or BL-4 agent. If it is determined that an exposure has occurred, the patient will be decontaminated and transported to Suburban Hospital by the responding EMTs. Patients wearing *CHEMTURION* suits will have their suit promptly sprayed with an appropriate decontaminant. When the patient arrives in the suit room, the suit will be removed. Once the protective suit is removed from the patient there is little, if any, likelihood of exposure of response personnel to an infectious agent.
3. Minimize the number of emergency responders and the amount of medical equipment entering the MCL. The patient(s) will be available for transport in either the suit room adjacent to the primary exit or outside the building.
4. Emergency response personnel, who have entered the MCL to assist the patient, will not exit with the patient but must go through appropriate decontamination procedures.
5. Hypoxia is an immediate threat unique to the MCL environment and is caused by inadequate oxygen supply to or distribution within the *CHEMTURION* suit. If this condition is suspected as a cause of loss of consciousness of a co-worker, the suit should be opened and the body adjusted to allow free breathing. This procedure should be done in an area in which it is unlikely that aerosols have been recently generated.

4.5 BOMB THREAT/INCIDENT

The chance of urban terrorism against an NIH facility is remote but a situation which nonetheless must be included in an Emergency Preparedness Plan. This section lists the sequence of steps to take in the event that either a suspicious package is discovered in or near Building 41A or a potential bomb threat concerning the MCL is received by phone. In both cases, remain calm and follow the instructions detailed in this section.

Engage the caller in conversation, be calm and, if possible, take notes to determine:

- exact location of the bomb
- source of the threat
- time of explosion

- background noises on phone
- peculiarities of the caller's voice
- gender and approximate age of the caller

If possible, have someone else listen on an extension or a speaker phone. Immediately upon termination of the call, and before using the phone to call the police, dial *69 and attempt to obtain the telephone number from where the threat originated.

Call the NIH Police 911.

Never touch a suspected bomb device; turn off all types of radios and transceiver equipment near the suspected area.

If building evacuation is necessary, leave in an orderly manner. Activation of the fire alarm system will not be used. The order to evacuate will be given by telephone or messenger.

4.5.1 Evacuation Procedures

1. Evacuate the building when given the order. If you can *safely* do so; secure all infectious materials in a refrigerator, freezer or incubator, close all animal cages and leave the cages in place in the room.
2. Exit through the normal entrance in an orderly fashion. In a life threatening emergency, a decontamination shower is not required. Emergency exits will not be used unless there has been a detonation.
3. Do not turn lights on or off. Leave the area as you found it.
4. Quickly scan your work area prior to leaving the building and report to the NIH Police any suspicious briefcases, bags, or packages, which appear to be out of place.
5. MCL occupants, who have exited the building without following the proper decontamination procedures, should remain isolated from other individuals and emergency responders since additional decontamination steps may need to be taken.
6. Personnel will evacuate the building to a designated marshaling area, at least 300 feet upwind from the facility, or where directed by the emergency response personnel. Personnel will return to the building when authorized by the Police Department IC.

4.6 EMERGENCY CALL LIST

FIRE/AMBULANCE/RESCUE/POLICE 911

ENGINEERING

NON-EMERGENCY CALL LIST

Occupational Safety and Health Branch

Occupational Medical Service

NIH Police

NIH Fire Department

Radiation Safety Branch

Environmental Protection Branch

Community Health and Pest Management

Buildings Maintenance Unit

Grounds Maintenance

LAB PHONE NUMBERS

MAIN Office	Rm 114
Suit Room	Rm 115
Sub-Basement	Rm 117
East Mechanical	
Guard Booth at 41A	

5.0 FLOOR PLANS

Removed for security reasons.

5.1 BUILDING 41A BASIC FLOOR PLAN

5.2 BUILDING 41A EMERGENCY PREPAREDNESS FLOOR PLAN

5.3 BUILDING 41A EMERGENCY GENERATOR POWER

5.4 BUILDING 41A ALARM SYSTEMS

5.5 BUILDING 41A GAS DECONTAMINATION

5.6 BUILDING 41A WASTE PROCESSING SYSTEM MCL

6.0 REFERENCES

1. Life Safety Code (NFPA 101) including Standards Referenced in Chapter 32 which include:
    a. NFPA 13 - Standard for the Installation of Sprinkler Systems.
    b. NFPA 30 - Flammable and Combustible Liquids Code.
    c. NFPA 45 - Standard on Fire Protection for Laboratories Using Chemicals.
    d. NFPA 70 - National Electrical Code.
    e. NFPA 72 - Installation, Maintenance and Use of Protective Signaling Systems.
    f. NFPA 72E - Standards on Automatic Fire Detectors.
    g. NFPA 80 - Standard for Fire Doors and Windows.
    h. NFPA 90A - Standard for the Installation of Air Conditioning and Ventilating Systems.
    I. NFPA 110 - Standard for Emergency and Standby Power Systems.
    j. NFPA 241 - Safeguarding Building Construction and Demolition Operations.
2. BOCA National Building Code including current supplements.
3. BOCA National Mechanical Code including current supplements.
4. Washington Suburban Sanitary Commission Regulations.
5. Model Energy Code.
6. OSHA Safety and Health Standards (29CFR 1910) including current revisions.
7. Maryland Stormwater Guidelines for State and Federal Projects.

8. American Society of Heating, Refrigeration and Air Conditioning Engineers (ASHRAE) edition of:

a. Refrigeration.

b. Equipment Handbook.

c. Fundamentals Handbook.

d. HVAC Systems and Applications Handbook.

9. Industrial Ventilation - A Manual of Recommended Practice by the America Conference of Government Industrial Hygienists.

10. Prudent Practices for Handling Hazardous Chemicals in Laboratories, National Research Council (19).

11. Additional NIH Standards.

a. Biosafety in Microbiological and Biomedical Laboratories, CDC/NIH, HHS Publication No. (CDC) 88-8395.

b. General Design Criteria for NIH Laboratory Use of Chemical Carcinogens.

c. NIH Safety Publications

I. The National Institutes of Health Radiation Safety Guide.

ii. The NIH Guidelines for Laboratory Use of Chemical Carcinogens.

d. Sprinkler System Design Criteria.

e. Waste Disposal at NIH.

f. Penetrations in NIH Buildings (Policy Memorandum #35).

g. Fire Protection (Policy Memorandum #34).

h. Shelving Heights in Laboratories.

I. NIH Guidelines for Controls.

j. Guide for the care and use of laboratory animals (NIH Publication 86-23)

7.0 APPENDICES

APPENDIX E-1 Request to Use NIH Maximum Containment Laboratory Checklist
APPENDIX E-2 Authorization For Entry Into The MCL
APPENDIX E-3 Internal Facility Telephone Numbers
APPENDIX E-4 OSHB MCL Training Checklist
APPENDIX E-5 CHEMTURION Extended Wear Model 35 Biological/Chemical Protective Suit
APPENDIX E-6 *CHEMTURION* Suit Dress Procedure
APPENDIX E-7 MCL Critical Systems Checklist
APPENDIX E-8 MCL PERSONNEL LOG-IN SHEET
APPENDIX E-9 Decontamination Shower Exit Procedures
APPENDIX E-10 Illness Surveillance Notice
APPENDIX E-11 Check-Out Procedures
APPENDIX E-12 Gas Decontamination Of Building 41a
APPENDIX E-13 Decontamination Airlock
APPENDIX E-14 MCL: Interior Checklist
APPENDIX E-15 Decontamination Autoclaves Standard Operating Procedures
APPENDIX E-16 Pest Management Program

Appendix E-1
Request to Use NIH Maximum Containment Laboratory Checklist

1. Scientific Director concurrence .	Yes	No
Abstract-Precis (limited to one page)	Yes	No
Protocol Background	Yes	No
Rationale	Yes	No
Protocol (detailed experimental design)	Yes	No
Discussion of Special Safety Issues	Yes	No
Statement of Potential Public Concerns	Yes	No
Personnel Listing and Background Information	Yes	No
a. Curriculum vitae(for scientific personnel)	Yes	No
9. Statement of demonstrated experience	Yes	No
10. Special language requirements	Yes	No
11. Other	Yes	No
Animal Study Proposal (as appropriate)	Yes	No
Statistical Considerations (with animal use)	Yes	No
Human Pathogen Registration Document (HPRD)	Yes	No
Recombinant DNA Registration Document (RDNA)	Yes	No
Radiation Safety Review (as appropriate)	Yes	No
Acknowledgment of Risks Statement	Yes	No

Appendix E-2
Authorization for Entry Into The MCL

This certifies that I have been informed of the potential hazards posed by the research to be conducted in the Maximum Containment Laboratory (MCL), Building 41A. I am familiar with the standard and emergency procedures described in the Safety and Operations Manual for Building 41A. I have been provided the OSHB required training and an OMS preplacement medical evaluation. I shall report immediately any known or suspected exposure to the agent or symptoms of infection with the agent to OMS and the MCL Occupational Safety and Health Specialist.

Attached are the following documents:

1. OSHB MCL Training Checklist
2. Human Pathogen Registration form, Animal Study Proposal and rDNA Registration form as applicable

Signature Date

_____ is authorized to enter and work in the MCL for the period_____to_____.

Chief, Occupational Safety and Health Branch Date

MCL Facility Manager/Occupational Safety and Health Specialist Date

(Original to OSHB, Copy to Principal Investigator & Employee)

Appendix E-3
Internal Facility Telephone Numbers

Not transmitted for security reasons.

Appendix E-4
OSHB MCL Training Checklist

SECURITY

1. Cardkey access
2. Hours of operation

ENTRANCE PROCEDURES:

1. Sign in, Building 41A Facility Control Center
2. Critical Systems Checklist
3. Disposable jumpsuit
4. Two-way radio head set (communication system)
5. Positive pressure suit

1) Preparation of suit
2) Check suit for defects
3) Donning of suit
4) Repairing suit
5) Operation of air lines

6. Airlocks

1) Explanation of interlock mechanism
2) Entrance to MCL through decontamination shower
3) Manual pull handle for decontamination shower
4) VHP Decontamination procedures

7. Protective boots
8. Internal Systems Checklist

HANDLING OF SUPPLIES/EQUIPMENT

1. Entry of material
2. Removal of material
3. Operation of Autoclaves
4. Transport of material within MCL
5. Storage
6. Packaging of material for shipment

COMMUNICATION OUT OF THE MCL:

1. Intercom
2. Telephone
3. Fax
4. Two-way radios

EMERGENCY PROCEDURES:

1. Location of alarms for:

1) Fire
2) Breathing air/HVAC
3) BSC failure
4) Status Panels (Other mechanical system failures)

2. Biological, chemical, and radioactive spill containment and clean up
3. Potential exposures
4. Health emergency

WASTE DISPOSAL

1. Medical Pathological Waste
2. Radioactive waste
3. Chemical waste
4. Multi-Hazard/ Mixed waste
5. Contaminated animals
6. Liquid waste

EXIT FROM MCL

1. Decontamination Shower

1) Normal operation
2) Emergency operation
3) Personal Shower (optional)

2. Decontamination airlock
3. Emergency Door (using the manual deluge shower)

REPORTING ACCIDENTS AND INCIDENTS TO THE FACILITY MANAGER AND/OR THE OCCUPATIONAL SAFETY AND HEALTH SPECIALIST.

1. Parenteral exposures
2. Needle sticks
3. Animal bites
4. Spills

The items on this checklist have been explained and/or demonstrated to me.

_____

Trainee

Date

_____

Trainer

Date

Appendix E-5
CHEMTURION Extended Wear Model 35
Biological/Chemical Protective Suit

The *CHEMTURION* Model 35 is a totally encapsulating biological/chemical protective suit. ILC Dover, Inc developed and manufactured this suit, and it has proven itself ideal for use in laboratory environments. This conformally fitting *CLOROPEL* suit with its 300E visor offers definite benefits where prolonged use and space limitations often cause worker fatigue. High volume air flow, made possible by multiple exhaust valves, supplies added cooling. These combined features provide comfort during extended wear thus increasing efficiency and productivity.

AIR SUPPLY: Umbilical-fed air is directed into a 1/4 inch NPT brass fitting. Positive air pressure in the suit is maintained by four exhaust valves, two located in the legs, and two located in the upper back. Each valve is protected by an integral splash cover.

VENTILATION: The suit air distribution system is metered to the arms and legs for cooling and to the hood spray bar for CO_2 wash, breathing, cooling, and defogging. The hood vent assembly contains an air noise suppressor to allow normal communication through the suit wall.

ENTRY: Suit entry is from the front.

CLOSURE: Outer extruded closure in conjunction with inner restraint zipper. Outer extruded closure made of *CLOROPEL* utilizes two (2) parallel sealing lips, providing an effective penetration barrier.

HOOD/VISOR: The hood contains press-polished optical grade 40 mil vinyl in the visual area. Internal easement permits head movement for 300 degrees of vision.

CONSTRUCTION: The suit is designed to minimize seams and permit user mobility without excessive suit shifting. The seams are dielectrically heat sealed. The suit incorporates molded wrist cuffs with mating rubber wrist rings for attachment of chemical protective gloves. The suit legs terminate in integral booties worn inside chemical protective boots.

MATERIALS: Suit--20mil light blue *CLOROPEL* (chlorinated polyethylene), Visor-40 mil vinyl.

SIZES: The suit is available in four sizes to fit 5'0" to 6'6". A belt is added to support optional equipment and to allow for a more conformal fit.

WEIGHT: Approximately 4 pounds.

DATA PACK: A data package is provided with each suit which contains pertinent information regarding operation, maintenance, testing, material compatibility, and critical applications criteria.

ACCESSORIES: HEPA Filters and suit air conditioners are available.

Appendix E-6
CHEMTURION Suit Dress Procedure

Before **each** use, the positive pressure suit **must** be inspected for defects. Everyone **must** have an **observer** while following the inspection procedure and donning the suit. The following procedure should be used.

When entering the outer change room, turn on the "Occupied" sign near the entrance door to allow privacy.

After entering the outer change room, remove all jewelry and street clothes, and store them in a locker. Lockers shall be cleaned once a week.

Don a disposable laboratory jump suit. This clothing is authorized for use inside MCL and will never be worn outside these areas. Only persons who have donned a laboratory jump suit may proceed into the suit area.

Select a pair of surgical gloves of the appropriate size in the supply area (between the personal shower and the suit room), and take them into the suit room.

Turn off the Occupied sign when entering the suit room.

Perform positive pressure inspection. Check the positive pressure suit to ensure that (i) there are no rips in the seams, (ii) the gloves on the suit do not have any holes or tears and (iii) there are no other apparent problems with the suit.

1. Lay the positive pressure suit on the table in the suit area.
2. Seal the four exhaust valves with vinyl tape.
3. Attach outer gloves to each arm of the suit, insuring that the gloves are in the proper position.
4. Close the zipper.
5. Close the exterior rubber seal over the zipper.
6. Attach the air line to the suit to pressurize it. Observe the suit closely, as it will inflate rapidly. Be prepared to remove the air line quickly.
7. Inspect the suit for defects, then deflate the suit. Deflation of the suit occurs with the opening of the exterior rubber seal.
8. Remove the vinyl tape from the valves.

Don surgical gloves and draw them up and over the cuffs of the laboratory jump suit. Tape gloves to the jump suit using vinyl tape.

Voice activated, two-way headphones shall be worn inside the suit to dampen sound and allow communication with others working in the MCL. These headphones are stored in the suit room.

Lay the suit down on the floor, straddle the suit at the "waistline", facing the feet.

Put one foot at a time into the feet of the suit.

Put the right arm into the right sleeve, put the hood on, than put left arm into the left sleeve.

Close the zipper.

Close the exterior rubber seal over the zipper. Everyone must have another individual check the outer plastic closure to ensure it is sealed from top to bottom.

Attach the air line to the suit. **Make sure the suit is connected to air lines at all times**, unless changing air stations.

Once the suit has been checked, disconnect the air line and enter the decontamination shower/air lock. The doors of the decontamination shower are **interlocking**. Therefore, one door must be closed and sealed before the opposite door can be operated. Allow the outer door to close and the gasket to reinflate, and then open the inner air lock door and proceed into the boot area.

Don protective footwear stored in the boot area and proceed into the MCL.

Sign the MCL interior checklist (see Appendix 7) and fill in the required items if no one else has done so. Record in the log the agents with which you are working.

Proceed with work in the MCL. Adhere to standard procedures that are used in the BL-4 laboratory, including the use of the BSC for **ALL** bench work with infectious agents (see BMBL for reference). Animal inoculations with infectious agents shall be done only in the BSC to reduce the risk of spreading infectious aerosols or infectious materials around the laboratory/animal rooms.

Appendix E-7
MCL Critical Systems Checklist

Check to indicate status is normal

GROUND FLOOR

A. Exterior Door Secure:

B. Alarm Panel, corridor:

Alarm Lamp Test

Pressurization Rm.114 ____ LET Sys ____ LCD Sys

Pressurization Rm116 ____ AHU #1 ____ AHU #2

Pressurization Rm.115 ____ Breathing Air Sys ____ Spares

Equipment door override ____ Shower door override

Ex Fan #1____ Ex Fan #2

C. Review computer proximity card history:
Comments_____

FCC Door Secure: _____ Air Lock Alarm Panel: _____

D. Autoclaves:

Large: Paper:_____ Cartridge:_____ Small: Paper:_____ Cartridge:_____

Weekly autoclave validation test, Date:_____

EAST MECHANICAL ROOM

A. Exterior door secure: _____

B. Supply and Exhaust Readings for the MCL: Enter PL; 4T the last two digits of the room #, then SW for supply or EW for exhaust for each room of the MCL; record readings in cfm.

14SW		15SW		16SW		17SW		10SW	
14EW		15EW		16EW		17EW		10EW	

C. Liquid Decontaminant Tank #9:

Level: _____ Comments_____

Firetrol, Inc. Pump Control Panel Mode:

Line Pressure Gauge (right of mix tank):_____psi. **Normal: 50 psi**

Pump Discharge Pressure:_____ psi. **Normal: 53 psi**

Tank: Auto_____, Manual Valve (under tank #8):_____

Inlet Valve: Tank: Open_____, Closed_____

Manual Drain Valve: Tank: Open_____, Closed_____ In-line Pressure: #1_____ #2_____

D. Travaini Breathing Air Compressors: Power On_____,

Pump monitors: Pump #1_____hrs. Pump #2_____hrs.

Line Pres_____ **Normal: 98-100 psi**

Water gauge: Pump #1:_____ Pump #2:_____ **Normal: 42psi**

Breathing Air Reservoir water drain (to be opened each Monday):_____

Carbon Monoxide Monitor:_____ppm. **Normal: 0.00 ppm**

E. UltraAir Compressor (Walz and Krenzer Doors): Compressed Air Gauge:_____ **Normal: 39-40psi**

Compressed Gases	Left Bank	Right Bank
Emergency Breathing Air	psi	psi
Carbon Dioxide (CO_2)	psi	psi
Argon	psi	psi
Nitrogen (N_2)	psi	psi

UPPER MECHANICAL SPACE

A. Air Drying Units Power ON #1_____ #2_____

Temperature: _____°F

B. Decontamination Deluge Tanks

North Tank #1:_____ gal. Comments_____

South Tank #2:_____ gal. Comments_____

WEST MECHANICAL SPACE

Exterior Door Secure:_____

	VFD (**Normal: 90-98%**)	Magnehelic	Static Pressure
Supply Fan #1	%	inches	inches
Supply Fan #2	%	inches	inches

PROCESSED WASTE: DAILY READINGS

Exterior Door Secure:_____ Manual Pressure Release Handle: Tank #1_____Tank #2_____

Pressure: Gauge: Tank #1_____ Tank #2_____

Normal: 0 PSI, during the cook cycle: 48 - 65 PSI

Temperature: **Normal: approximately 80°F**

Gauge: **In the cook cycle - Tank #1 or #2: 250-264°F**

Tank #1_____ Tank #2_____ Tank #3_____ Tank #4_____

Tank #5_____

Water Level:

Normal: High Water Level (HWL): 63" or less. In the cook cycle, the HWL is 63" - 73"

Low Water Level (LWL) - 3"

Tank #3, #4, and #5: HWL - 45" - 48"; LWL - 3-5"

Gauge: Tank #1_____ Tank #2_____ Tank #3_____ Tank #4_____ Tank #5_____

Temperature Chart: Date of Change:_____ Filed:_____

REMARKS:_____

_____

_____

_____

Signature:_____Date:_____Time:_____

MCL: Critical Systems Checklist - Narrative

The purpose of this checklist is to monitor and maintain a record of the status of Containment and Life Support features of the MCL. The Critical Systems Checklist is normally completed by OSHB personnel before 8:00 AM on regular workdays. On holidays and weekends, the checklist shall be completed by MCL Staff before anyone enters the MCL. Most observations vary little from day to day, so be sure to be alert for anything unusual. If any questions arise, contact Building 41A Maintenance (402-3039), South Building Maintenance (6-6484) or OSHB (6-2346).

FACILITY CORE CENTER CORRIDOR

1. Door secure - A check indicates the single north entry door was checked and found to be secure.
2. The alarm panel in corridor 101 is located inside and to the left of the entry door. A light on this panel indicates an alarm situation. Indicate with a check if there are no apparent alarms. Any alarms shall be reported immediately to Building 41A Maintenance and OSHB. No one shall enter the MCL until the source of the alarm has been resolved.
3. Review computer proximity card history for the previous evening and note if there were any abnormal entries.
4. Autoclaves
5. A check indicates autoclaves were checked. Autoclave is in standby mode prior to 7:00 am. If autoclave is running - indicate in the proper blank. If the steam is on and the autoclave is open, contact the Occupational Safety and Health Specialist to determine status. The supply of autoclave paper should be sufficient for the day.

EAST MECHANICAL AREA

1. Door secure - A check indicates the exterior door and the interior door to the east mechanical area was found to be secure.
2. Use the computer in the east mechanical area office to record the supply and exhaust fan readings for the MCL. For example, enter Shift P, L. Enter 4T14SW to get the supply fan reading for room 114. Enter 4T14EW to get the exhaust fan reading. To print the CFM for the 41A, enter PL4T*W, hit enter, print. To print the pressures for 41A, enter 4T*P, hit enter and print.

LIQUID DECONTAMINATION TANK

Indicate with a check if level of Tank #9 is between the low and high level alarms. Comments, note anything unusual e.g., no volume change, that may indicate a malfunction. Contact South Building Maintenance (6-6484) or Building 41A Maintenance (2-3039), if there are any questions or irregularities noted. Read and record status of control panel and valves. A check indicates condition. Note that manual drain valve is closed except during drain mode.

DECONTAMINATION SHOWER TANK INSTRUCTIONS

ALL PERSONNEL SHALL WEAR A FULL FACESHIELD AND GLOVES WHEN PERFORMING THIS PROCEDURE. THE APPROPRIATE PROTECTIVE EQUIPMENT IS LOCATED NEXT TO THE TANK LOCATED NEXT TO THE TANK.

1. Check level of decontamination solution.
2. Manually add appropriate decontamination solution to the Mix Tank. The measurement of water is automatic.
3. Set the automatic timer to control the operation of the mixer.
4. Ensure lid on the Mix Tank is securely closed.
5. Complete Decontamination Shower Mix Tank record on the Critical Systems Checklist.

An alarm is activated when the volume falls below gallons. If it approaches this level please alert the Occupational Safety and Health Specialist on call. The in-line fluid pressure should have a reading of 10 psi., notify Building 41A Maintenance (2-3039), if it is more than ±5 psi. The line pressure gauge should have a reading of 50 psi. The pump discharge pressure (above the pumps) should have a reading of 53 psi.

6. Travaini Breathing Air compressors

Check and record that the power switches are ON. These power switches are red and black with arrows on them. The arrows should be in an Aup@ position. There is a red strobe located on the top of the Travaini Breathing Air control panel. This strobe should not be on at any time. The strobe will be on only in the event of a malfunction in the breathing air system. **No one shall enter the MCL until this malfunction has been located and resolved.** Record pressures from tank pressure gauge to the right of water tank. Normal is 98 - 100 PSI. Check and record water gauge pressures on each pump. The normal reading is 42 psi; if a pump is running, the reading will be 36 -38 psi. The black bypass valves, located above pumps 1 and 2 should be checked. This valve should not be full open, but just slightly open. Record the number of hours each pump has been run, the monitor is located on compressor control panel. At 1000 hours, pumps must be greased with Lithium grease (one shot only). At 3000 hours, the filters on each pump must be changed. When the water tank pressure reaches 91 psi, one pump will turn on. The pump will run for ten minutes, and then the second pump will run. This alternating schedule is normal for these pumps. The breathing air reservoir is located behind the Travaini Panel. At the base of the reservoir, there is a green release drain. This drain shall be opened each Monday until all the water has drained out of the system. **The carbon monoxide monitor should read 0 - 1 part/million. 10 parts/million is unsafe. No one should enter the MCL if the monitor has a reading of 10 parts/million. The carbon monoxide monitor should be calibrated every 6 months.** Check and record pressures of reserve

emergency cylinders in the A-Bank (left) and B-Bank (right) on the monthly record attached to the tanks. If a reading varies more than 50-100 PSI from the normal reading, contact Building 41A Maintenance. Record pressure (PSI) on daily checklist.

7. Read and record the pressure (PSI) of the UltraAir Compressor air gauge (Walz and Krenzer Doors)
8. Read and record individual pressure for CO_2, argon, and N_2 cylinders. If either tank is <300 PSI, contact Building 41A Maintenance.

Upper Mechanical Space

1. The power should be ON for both air drying units, indicate with a check .
2. Read and record the volume of liquid in each tank. Record on the daily Critical Systems Checklist.

West Mechanical Space

1. Indicate with a check if the exterior door is secure.
2. On the VFD Panel, read and record the supply fan efficiency in %.
3. Read and record the magnehelic and static pressure gauges for both supply fans.

Process Waste System

1. The Process Waste System is a fully automatic waste processing system. Under normal operating conditions, none of the controls shall be changed. The pressure gauges for tanks 1 and 2 are located on top of each tank. The manual pressure release handle is located on the pressure line above each tank. This handle should always be in the **closed** position.
2. The temperature gauges for tanks 1 and 2 are located behind the tanks, at approximately the three-quarter point of the tanks. The temperature gauges for tanks 3, 4, and 5 are located at the rear base of the tanks.
3. The water level gauges for all the tanks are located on the west wall on the Powers Process Control panel. The water level shall be recorded in inches of water.
4. The Honeywell Temperature Charts shall be (i) changed each week, (ii) dated and signed on the reverse of the chart, (iii) and filed in the Facility Core Center.

Remarks

Note here any deviations or comments for the record. Sign, noting date and time.

Appendix E-8
MCL PERSONNEL LOG-IN SHEET

Date/time entry	Signature	Activity	Date/time exit	Signature	Comments

Appendix E-9
Decontamination Shower Exit Procedures

I. Description of Decontamination Shower Airlock

A. Stall

1. Shower jets projecting from the four corners of the shower stall and overhead.
2. Shower basin with an outlet drain, which leads to the Process Waste tanks located in the sub-basement of Bldg. 41.
3. Green start button located on the south wall
4. Manual deluge shower head and handle

B. Shower doors

1. The doors are air gasketted, stainless steel, and interlocking.
2. The gasket is disengaged by pushing down on the door handle.
3. The exterior decontamination shower door may not be opened unless a decontamination cycle is run.
4. In the event of an emergency, there are manual overrides of the interlocking doors located in the interior of the shower and in the suit room adjacent to the door. These manual overrides are activated by gently pulling down on the lever. The release of air from the gasket can be heard as it deflates.

C. Cycle

1. Cycle is activated by pushing the green Astart@ button.
2. Pre-mixed decontamination solution sprays through the shower jets for the first half of the cycle followed by a clear water rinse.
3. Water is tempered to 80° F.

II. Decontamination shower procedures for exiting the MCL

A. Remove boots and leave in boot area, enter the decontamination shower, and close shower door. Door will automatically lock.

B. Connect to airline.

C. Push “start” button to activate the cycle.

D. During the decontamination cycle, lift arms above the head and turn 360 degrees; physically scrub the suit with gloved hands.

E. After the water stops, open the exterior door and exit the shower.

F. Close the exterior door, to allow the next individual to exit the MCL.

G. Remove the positive pressure suit, spray the interior of the suit with the alcohol solution provided in the suit area.

H. Hang the suit on the overhead hooks in the suit area.

I. Turn on the "Occupied" light (located on the east wall of the suit room) for privacy. Enter the inner change room, dispose of the jump suit in the designated waste container.

J. Take a personal shower if desired. Eyeglasses worn in the MCL must be washed during the personal shower.

K. Redress, place the towel in the hamper in the outer change room, and exit the dressing room. Turn off the "Occupied" sign.

L. Sign out on the personnel log and record any condition requiring OSHB attention.

III. Malfunction of Liquid Chemical Decontamination System

A. Report to the MCL Occupational Safety and Health Specialist if shower jets are not functioning.

B. When the decontamination shower cycle does not function properly, the manual decontamination system may be activated. Use the pull handle on the deluge shower.

1. First dowse the suit thoroughly with disinfectant.
2. Scrub suit with gloved hands.
3. Repeat step.
4. Exit the shower and continue with standard procedures.
5. Inform OSHB as quickly as possible.

Appendix E-10
Illness Surveillance Notice

Employee Name: _____

Address: _____

_____

Phone: _____

Symptoms: _____

_____

_____

_____

PI Signature

Chief, OSHB: _____

Phone(H): _____ Phone(W): _____

Pager: _____

Principal Investigator: _____

Phone(H): _____ Phone(W): _____

Pager: _____

Facility Manager: _____

Phone(H): _____ Phone(W): _____

Pager: _____

Occupational Safety and Health Specialist:

_____

Phone(H): _____ Phone(W): _____

Pager: _____

Occupational Safety and Health Specialist:

_____

Phone (H): _____ Phone(W): _____

Pager: _____

Safety Operations Section, OSHB, Phone: 496-2346

Occupational Medical Service, Phone: 496-4411

NIH Security, Phone: 496-5685

Fire/Ambulance, Emergency: 911 (on campus)

Appendix E-11
Check-Out Procedures

Name (Investigator) Date

1. Remove and properly store all Biological Material from refrigerators and/or freezers.

Signature, Facility Manager or Occupational Safety and Health Specialist — Date

2. Empty locker

Signature, Facility Manager or Occupational Safety and Health Specialist — Date

3. Removed from Building 41A proximity card user list

Signature, Facility Manager or Occupational Safety and Health Specialist — Date

4. If Visiting Scientist at the NIH, return NIH identification.

Signature, Facility Manager — Date

Appendix E-12
Gas Decontamination of Building 41a

Gas decontamination of the MCL of Building 41A is necessary at the end of a research project or annually, whichever comes first. After decontamination has been completed; (i) preventive maintenance will be performed on all equipment in the MCL; (ii) HEPA filters shall be tested and changed if necessary; (iii) all building systems maintenance (eg. HVAC, plumbing, electrical, etc.) to the building shall be performed at this time.

Pre-decontamination Procedure

A. Empty refrigerators and mechanical freezers. Turn off (unplug) and defrost freezer, mop up water, and prop doors open. Unplug icemaker, turn off water valve, open door, and allow to drain.

B. Surface decontaminate delicate equipment (e.g., microscopes) and move to the VHP decontamination airlock if it is to be removed from the MCL.

C. Dispose of all animals and animal food in the MCL.

D. Empty laboratory of unused labware and laboratory supplies and place them in the autoclave.

E. Unplug centrifuges, incubators, and other fixed equipment. Leave doors open.

F. Pour appropriate decontaminating solution into all sink traps and floor drains.

G. Open all cabinet doors and drawers.

H. Arrange for maintenance of laboratory equipment on maintenance contracts and all operating systems of the MCL during shutdown period.

I. If radioactive materials were used, the following clearance procedures must be performed before the shutdown period:

1. All radioactive materials must be removed and secured in another properly posted lab or disposed of through the radioactive waste disposal service.
2. All items which are potentially contaminated with radioactive materials, e.g., refrigerators and centrifuges, shall be decontaminated and removed to another posted laboratory or stripped of all "Caution Radioactive Material" labels if radioactive materials are removed.
3. Empty radioactive waste containers must be decontaminated and moved to another posted laboratory or removed by the radioactive waste disposal service.
4. Any area of contamination at levels greater than 220 dpm/100 cm^2 (22 dpm/100 cm^2 for alphas) must be decontaminated until an appropriate survey indicates less than allowable limits.
5. Clearance must be scheduled by contacting the RSB. RSB will review the survey performed by lab personnel, spot-check the MCL laboratory/animal suite, and if no problems are found, remove the "Caution Radioactive Material" sign at the entrance to the MCL and replace it with a RSB clearance certification.
6. As long as an RSB clearance certification is posted at the entrance to the MCL laboratory/animal suite, no radioactive materials can be used or stored in that laboratory/animal suite. When radioactive materials are to be used in the laboratory/animal suite again, lab personnel must notify the RSB to remove the clearance certification and post the laboratory/animal suite with a "Caution Radioactive Material" sign.

Procedure

A. Calculate the total volume of the area for gaseous decontamination.

1. Calculate the amount of paraformaldehyde to be used. Multiply the area volume (cubic feet) by 0.3 to determine the amount of paraformaldehyde/grams. Total volume of laboratory area is 10,650 cubic feet. The required amount of paraformaldehyde is 3195.0 grams.

2. This amount of paraformaldehyde will provide an equivalent formaldehyde concentration of 0.8% by weight or 10,000 parts per million by volume in air.

   NOTE: Do not to use a greater amount of paraformaldehyde than is required. An excess amount can cause the formaldehyde to polymerize on surfaces as a white powder which is combustible in this form.

3. Calculate the amount of ammonium carbonate necessary to neutralize the formaldehyde gas. The ratio is 1:1.1 of paraformaldehyde to ammonium carbonate by weight. The required amount of ammonium carbonate is 3514.5g.

B. Assemble necessary equipment and materials.

1. Obtain the following:

   a. 10 electric frying pans

   b. Water

   c. 24 spore strips

   d. 5-10 extension cords

   e. Pre-measured paraformaldehyde (See attached floor plan).

   f. Pre-measured ammonium carbonate (See attached floor plan).

   g. 5 sheets aluminum foil

D. Decontamination of MCL

1. Clean all surfaces in the MCL with the appropriate decontaminent and place spore strips throughout the facility.

2. The electrical power controls for the dedicated decontamination sockets are in the West Maintenance Area. These controls are (i) marked with the corresponding room numbers, (ii) the toggle switches have three positions, (iii) when the toggle switch is in the center position, these dedicated sockets are without electrical power, and (iv) these switches will be maintained in the off position when not in use for decontamination purposes.

3. Place and connect the pans to the electrical sources following the attached floor plan which indicates the toggle positions with the corresponding sockets.

4. Close the isolation dampers of each room.

5. Open the pass through doors between the laboratory/animal rooms.

6. Fill the floor drains with decontamination solution.

7. Spread the pre-measured paraformaldehyde in the designated electric frying pans and add water. Spread the pre-measured ammonium carbonate in the remaining electric frying pans and cover with aluminum foil.

8. Set the thermostat control of the electric frying pans at 450Ef (maximum operating temperature).

9. Inflate the gaskets on the doors.

10. Turn on electrical power to paraformaldehyde frying pans, and allow to heat until all the paraformaldehyde cooks off.

11. “Bump" the blower in the BSC for 3 to 5 seconds four times during the paraformaldehyde cook-off (at 25%, 50%, 75% and 100%)

12. Turn off electrical power to paraformaldehyde frying pans. **Allow overnight contact time.**

13. Turn on electrical power to ammonium carbonate frying pans, allow to heat until the ammonium carbonate cooks off. **Allow overnight contact time.**
14. Open isolation dampers of each room. Allow the rooms to vent overnight.
15. Collect and incubate spore strips.

Appendix E-13
Decontamination Airlock

I. Vapor Phase Hydrogen Peroxide (VHP) Decontamination Procedure

A. Prior to Decontamination Date:

1. Determine suitability of material/equipment. [Note: The VHP is suitable for clean surface decontamination of objects. It there is any protein or chemical material on the surface of the object, decontamination of that area may be ineffective.]
2. Notify MCL Occupational Safety and Health Specialist of intent to decontaminate material using the VHP (using form, Appendix 13a).
3. Review VHP protocol with the MCL Occupational Safety and Health Specialist.
4. Consult AMSCO VHP Equipment Manual to determine cycle parameters.

B. Day 1

1. Record decontamination information on VHP Log.
2. Move material/equipment to be decontaminated into the VHP room/airlock. Do not block outlets.
3. Place spore strips: near door, on floor, and on material/equipment to be decontaminated.
4. Close inner door and tape with "DO NOT ENTER" yellow tape. **LEAVE ROOM SEALED, WITH DAMPERS CLOSED OVERNIGHT.**
5. Run decontamination cycle.

C. Day 2

1. Open airlock dampers and allow room to ventilate for 3-4 hours.
2. The exterior airlock door shall only be opened by the MCL Occupational Safety and Health Specialist.
3. Open exterior airlock door, recover spore strips.
4. Incubate spore strips per standard protocol. Do not remove materials or permit work to progress in the decontamination chamber until no growth has been verified for at least 24 hours.
5. Upon completion of spore strip testing, remove equipment through exterior airlock door. Close exterior door, the gasket will re-inflate. If any service work is to be performed on the equipment in the decontamination chamber, equipment should be wiped off prior to servicing.
6. Arrange for OSHB personnel to remove "DO NOT ENTER" yellow tape.

Appendix E-14
MCL: Interior Checklist

Check to indicate status is normal:

Restroom supplies: Toilet Paper: _____ Paper Towels: Soap:_____

Cleaning Products: _____

Directional Airflow: _____

Emergency Door Secure:_____

1. Exhaust filters _____ (date of last change on filters) (Change Annually for lab only work. If using animals, animal care staff will advise)

2. Autoclaves Large Small

3. Waste Containers (empty daily)

4. Lab Supplies:

5. Facsimile:
   Paper
   Toner

Fax this list to the facility core center: 0-1377

Appendix E-15
Decontamination Autoclaves
Standard Operating Procedures

1. Please contact the MCL Occupational Safety and Health Specialist prior to operation of the autoclave to coordinate the removal of the MPW boxes after sterilization of the waste material. Remove and inspect the chamber strainer grate. Clean the strainer before each autoclave run. Check printer for adequate paper; if print is difficult to read, please change cartridge. Multiple Diak tubes should be included with each cycle.
2. Load the autoclave, arrange multiple Diak tubes with waste material, and close the door.
3. The cycles are pre-programmed. Consult programming list posted on autoclaves. Select the desired cycle for the material to be autoclaved. Use the gravity cycle for decontamination loads containing small amounts of liquids. The liquid cycle is for large volumes of liquids and requires a longer time for the pressure to return to normal after sterilization. The vacuum cycle is appropriate for dense material, e.g. feed, and animal litter. NOTE: Waste decontamination cycle is 90 minutes for BL-4. Longer runs may be required for large volumes of waste, particularly animal waste. If in doubt, contact the MCL Occupational Safety and Health Specialist in the Building 41A Facility Core Center, or OSHB (6-2346).
4. When the cycle is complete, open the clean side door and remove the sterilized materials. Remove the printed tape-record of the run and place it with the autoclaved materials. NOTE: This function is normally handled by MCL personnel. It is imperative that the printer tape is placed with the autoclaved materials to show that run parameters have been met. The Diak tubes should be examined to ensure that the autoclave was operating properly.
5. Close the clean side door so the autoclave can be opened from inside the MCL to add a new load. Place autoclaved material in MPW boxes, stamp each box indicating sterilization of the box.
6. At the end of the day, the autoclave will automatically go into standby mode. The autoclave has been pre-set to turn off at 7:00 pm Monday-Friday.

   DO NOT TURN OFF THE MAIN POWER SWITCH TO THE AUTOCLAVES.
7. Once a week, multiple spore strips should be used in the autoclave to monitor the efficacy of the autoclave cycles.

Contact the MCL Occupational Safety and Health Specialist to obtain:

Printer paper or cartridges, waste pick-up information, autoclave pans or bags and biological monitoring material.

Appendix E-16
Pest Management Program

Background

Integrated pest management (IPM) is an interdisciplinary approach to controlling pests. Traditional pest control programs (i.e., extermination) rely primarily on preventive or corrective applications of pesticides. IPM emphasizes managing the environment in order to make it least supportive of pest activity. Detection of pests and pest activity, along with facility conditions and operational practices, are part of the ongoing assessments to identify conditions conducive to pests performed at each service.

Scope of Services

A. Frequency of Service

IPM services will be performed weekly in the mechanical spaces, locker room, building exterior and support areas. At the end of three months, the pest management data will be reviewed and, with concurrence of the Facility Manager, the frequency of service may be decreased to twice per month. This decision will be based on the number of pests trapped or sighted, conditions in and around the facility, our ability to effectively monitor the facility and the requirements of the facility personnel. The frequency of service may vary somewhat depending upon the season and programmatic changes inside the facility.

B. Areas to be Serviced

Monitors (glue traps) will be placed in the following areas:

- The mechanical rooms
- The locker room
- The Facility Core Center
- The sub-basement of Building 41

C. Service Procedures

1. Pest management personnel will meet with the Facility Manager prior to performing any IPM services.
2. IPM services will consist of the following:

a. Review of logbook and pest management records

b. Discussion(s) with Facility Manager and other personnel concerning pest management issues.

c. Monitoring of all accessible areas for evidence of pests or conditions conducive to pest activity. Monitoring is the fundamental activity in any IPM program. Monitoring is a means of collecting quantitative information on pests and qualitative information on facility conditions and personnel practice that may promote pest activity. The IPM service schedule, training programs, service reports and recommendations, and pest control tactics are all based upon analysis and evaluation of monitoring results. Monitoring is usually performed using traps, visual surveys and interviews with facility personnel.

d. Inspection of all pest management monitoring devices in and outside of the facility. All monitoring devices will be checked, marked with the date and initialed by the pest management personnel at each service. Monitors that contain insects, are dirty or no longer effective will be replaced.

e. Completion of a pest management survey report outlining all services performed, and including recommendations for corrective pest management actions regarding personnel practices and facility repair. A pest management survey of the MCL will be performed after each facility decontamination.

f. A written report of all observations and recommendations will be submitted to the facility manager after each survey.

Survey of the Maximum Containment Laboratory

The MCL will not be surveyed on a routine basis. Pest management personnel will be available to monitor the interior of the containment upon the requested of the facility manager. Live or dead insects or evidence of pest activity (i.e., casts insect skins, feces, body parts, etc.) will be used as thresholds to determine if intervention by pest management personnel is necessary. Often, monitors or other trapping devices can be placed by facility personnel as an interim measure to determine if additional pest management action is necessary.

Emergency Service

In the event of a pest related emergency, PMU staff can be contacted by telephone and pager. A list of Pest Management Unit personnel, pager numbers and home telephone numbers will be provided to the Facility Manager. In the event of a pest related emergency, PMU personnel can be contacted to advise on pest management questions or perform IPM services.

Pest Management Equipment

The following pest management equipment will be used as part of the pest monitoring and control activities in Building 41A:

1. Sticky traps for insects and nuisance arthropods
2. Sticky traps for flies
3. Glue boards for rodents
4. Live traps of rodents (Ketch-all, Tin Cat, Hav-a-Hart traps, etc.)
5. Insect light traps - installed both permanently and temporarily to monitor for and control flying insects
6. Hydramethylnon - solid formulation of an insecticide for use against cockroaches, ants, crickets, etc.
7. Avermectin - solid formulation of an insecticide for use against cockroaches, ants, crickets, etc.
8. Caulk - silicone/acrylic caulks will be used in isolated areas to exclude pests
9. Spring Traps - used to control rats and mice
10. Stored Product Pest traps - these are glue traps baited with insect and insect pheromone attractant. They will be used in the animal feed storage area.

The Facility Manager will be apprised of the placement of all pest management monitoring and control devices. Live traps and glue and snap traps for the control of rodents will only be used in areas of known infestation. Once placed, the traps will be checked daily to ensure the humane treatment and prompt removal of any trapped rodents. The application of insecticides will also only be performed in areas of known infestation. The use of liquid or aerosol formulation insecticides will be restricted to unique application where other control tactics are infeasible or not effective. Liquid or aerosol formulation insecticides will only be applied after consultation with the Facility Manager.

Personnel and Facility Related Issues

Given the high level of sanitation and maintenance associated with the operation of this facility, the opportunity for infestation of this building by insect or rodent pests is minimal. However, the potential for incidental ingress, particularly by ants, night flying insects, crickets, etc. and insect pests from home or laboratories. The following procedures should be used to minimize this problem:

1. Empty all lockers at the end of each week.
2. The Facility Manager should have a master key for all locks used on lockers.
3. Eating is not permitted anywhere in Building 41A.
4. Animal feed should be stored in Building 41A only for a limited amount of time, (i.e., two weeks). If it is not consumed within this time frame, it should be disposed of. This is to prevent stored product pests from becoming a problem in the facility. Also, keep the minimum amount of feed required on hand and store all feed and bedding products in cleanable, sealable plastic containers.
5. All security lighting should be directed at the building from the surrounding area, not mounted on the building.
6. Sodium vapor lights should be used outside the building.
7. Do not landscape with ground covers, such as ivy or pachysandra, or dense shrubs/bushes.
8. The waste should be disposed of in a vertical compactor to deter rodent activity. The compactor should be small enough to facilitate frequent emptying, preventing the accumulation of waste.
9. The seals on both roll-up over head doors should be checked frequently to insure proper seal at the ground.
10. The conduit connecting Building 41 with Building 41A should be treated with a bird deterrent device.
11. Electric insect traps should be installed at all entry/exit doors, and throughout the facility, where practical.
12. Better seals are needed around both roll-up overhead doors and especially at the bottom of the doors. There is also a gap at the top of the door that must be sealed.

Part 2 – Decontamination Equipment and Procedures

Decontamination Equipment and Procedures

INTRODUCTION

Containment of potentially infectious agents in laboratories is managed by laboratory design, the use of good laboratory practices, chemical germicides, and equipment such as biological safety cabinets and autoclaves.

All contaminated materials must be decontaminated before disposal or cleaning for reuse. This includes laboratory surfaces, rooms and equipment, which may require decontamination prior to servicing. It is the responsibility of each person who works in the laboratory to ensure that proper decontamination procedures are followed and that containment is not breached.

The method chosen is determined by the nature of the material to be treated, i.e. if it is disposable, is adversely affected by heat, cannot be penetrated by steam, etc.

AUTOCLAVES

Autoclaves are instruments which maintain saturated steam at high temperatures and under pressure. They are used to sterilize laboratory equipment and materials by destroying potentially infectious agents. A typical autoclave cycle of 15 minutes at 121EC and 15 psi is usually sufficient to kill the most heat resistant microbiological agents, i.e. bacterial spores.

The autoclaves that are used at the WNCBED are all Getinge/Castle models, either M/C 3522, M/C 3622 (gravity) or M/C 3633 (vacuum) autoclaves. There are three types of autoclave cycles: unwrapped, wrapped (employs vacuum pulsing to condition a load prior to processing), and liquid (slow exhaust times). Wrapped and unwrapped cycles are run for solid materials. Liquid loads require slow exhaust times to avoid boiling during pressure reduction. A description of load types and cycle settings should be prepared and posted for each autoclave.

STANDARD AUTOCLAVE CYCLES

Unwrapped cycle

The unwrapped cycle employs a gravity assist during the conditioning phase and the exhaust phase (M/C 3633) or in the exhaust phase only (M/C 3522 and M/C 3622) to displace air.

Wrapped cycle

The wrapped cycle employs a pulse during the conditioning phase of the cycle. This effectively cycles the temperature and allows for the removal of any air pockets that may arise. This cycle also employs a gravity-type exhaust.

Liquid cycle

The liquid cycle employs a gravity assist during conditioning. However, during the exhaust phase, the chamber pressure is gradually decreased. This prevents a vacuum and subsequent boiling of any liquids present.

SAFETY PRECAUTIONS

- Wear protective clothing (ie. autoclave gloves and apron) when removing the contents from the autoclave.
- Autoclaves and contents present severe burn hazards. Standing away from the door minimizes the risk of burns due to evacuation of steam or fluids from the autoclave.

- Autoclaves operate under pressure. To prevent a burn and physical shock hazard never attempt to force autoclave doors open before the end of a cycle or when the jacket pressure is greater than zero.

BIOLOGICAL INDICATORS

Biological indicators are used to develop the processing times for typical loads and monitor the efficacy of the decontamination process. Efficacy monitoring must be done at least once per week in BSL-2 areas, depending on the frequency of use of the autoclave, and with **each load** coming out of BSL-3 and BSL-4 areas. Records should also be kept of the time, temperature and pressure of the load by attaching the autoclave print-out to the log book file. Thermocouples, placed at the centre of the load, may also be used to monitor the internal temperature of the load. These may be used in conjunction with a biological indicator.

The basic procedure for **efficacy monitoring using biological indicators** is the following:

1. Place biological indicator (Getinge/Castle Biosign biological indicators (spores/indicator: 10^4 *B. stearothermophilus* and 10^6 *B. subtilis var. niger*, 3M Attest Rapid Readout biological indicator or Raven Prospore biological indicators (liquid loads)) in the center of a typical load (each different type of load should be tested separately).
2. The load is processed according to standard operating procedures, taking into account the lag time necessary for the internal temperature in the center of the load to reach the sterilization temperature (this time will vary depending on the nature of the load being sterilized); even though the spores of *B. stearothermophilus* are killed when exposed to 121EC for 15 minutes, the total cycle time depends on the load.
3. After completion of the cycle, the autoclave is opened and the biological indicator is removed.
4. Biosign indicators are taken to the Biosafety lab and incubated at 55EC, along with a positive control that did not go through the autoclave process; they are examined at 24- and 48 h. for growth; a color change from red to yellow indicates growth and sterilization failure (i.e., parameters of time and/or temperature have not been met in the test indicator).
5. Absence of growth in the test indicators signifies that sterilization of the load was achieved, representing a 4-6 $\log_{10}$ reduction in *B. stearothermophilus* spores.
6. Attest Rapid Readout biological indicators are taken to Biosafety lab and incubated at 60C. (Note: Attest Auto-reader must be calibrated with a STERILIZED, non-incubated Attest Biological Indicator). A positive control of the same lot number that did not go through the autoclave process must be included.
7. Absence of fluorescence in 3 hrs indicates that sterilization was successful.
8. Prospore biological indicators are taken to biosafety lab and incubated at 56C along with a positive control that did not go through the autoclave process; they are examined periodically for growth.
9. Growth is indicated by a color change from purple to yellow. This indicates a failure of the sterilization cycle.
10. Failure to achieve sterilization may be due to improper loading or overloading of the autoclave (i.e., the center of the load failed to achieve sterilization temperature), or insufficient sterilization time; the process should be repeated until the necessary loading configuration and sterilizing time have been determined; this time and load configuration should be used for all subsequent cycles for that type of load.

NOTE: Chemical indicators for steam, time and temperature are useful for day-to-day monitoring but must not be used as an indicator of sterility. Biological indicators are required to indicate sterility; labels and tapes that only indicate the attainment of temperature, not its duration, are not recommended.

MAINTENANCE

Daily

1. Clean sediment screen. Remove the sediment screen from the chamber drain and clean thoroughly. It can be removed and replaced without tools.
2. Clean exterior surfaces. Routinely clean the exterior surfaces with Tec Surf (Castle Part No. 47104) or other mild cleaning agent. Do not use strong or harsh solutions which may damage painted surfaces.
3. Clean accessories. Clean loading carts, racks, shelves, baskets, trays, etc. with a mild detergent and water solution.
4. Clean chamber interior. Clean interior surfaces of sterilizer chamber with a mild detergent and water solution. Rinse with water.
5. Clean material handling carts. Clean material handling carts with a mild detergent and water solution. Rinse with water.

Monthly, 3-Month, Semi-Annual, Annual

Routine maintenance will be the responsibility of the Operations & Maintenance (O & M) group. The EHS group will perform a thermocouple test on all autoclaves annually following scheduled maintenance or following unscheduled repairs.

When Required

1. Replace ink cartridge. When replacing the ink cartridge, care should be taken not to twist or bend the pen arm. A deformed or bent pen arm could record a false reading. Removal: Hold the pen arm with one hand and pull the old pen straight off the end of the arm. Replacement: Place the new pen cartridge on the top of the pen arm (pen downward and outward) and push the cartridge into the remaining clip. Be sure the pen fits into the notch in the end of the arm.
2. Replace door gasket(s) (M/C 3522).
   a) Remove the sterilizer front trim panel, which is secured with 4 screws. Remove the hex nut and rubber door stop bumper from the lower frame cross member.
   b) Allow sterilizer to cool before removing the gasket. Remove the gasket by pulling it out of the head ring gasket groove, with the door at the fully lowered position.
   c) Remove any sharp edges of burrs from the gasket groove that may damage the gasket.
   d) Clean the gasket groove with alcohol to remove any foreign material that may have collected in the groove.
   e) Ensure the replacement door gasket is clean. Place the gasket splice at the top centre of the gasket groove and press it into place. Divide its length evenly and press the gasket into place at the bottom center of the gasket groove. Press the gasket, evenly divided, into the centre of the gasket groove on the sides, and then the four corners. Make sure the gasket is fully recessed to prevent damage by the door and replace the rubber bumper and front trim panel.

PASS-THROUGH CHAMBERS

The laboratory is equipped with a number of pass-through chambers which are designed to enable to laboratory staff to safely transfer materials from the clean side to the dirty side without compromising the containment capabilities of the area. It is imperative that the sequence of events be followed to ensure directional airflow is maintained during the procedure.

TRANSFER OF MATERIALS (CLEAN TO DIRTY)

Operation of the pass-through is a two-person operation, one on the dirty side and one on the clean. The controls for the ventilation adjustments are located on the dirty side and must be configure properly prior to opening of the clean side door.

1. Establish voice communication between the two sides of the pass-through.
2. Prior to opening of the clean side door, close the atmospheric vent (ball valve, #1), which enables equalization of the pressure when the pass through is not in use and the open the ventilation valve (exhaust duct, #2) to provide additional negative air flow.
3. Open the clean side of the pass-through and place the materials into the chamber.
4. Reseal the clean side door and notify the individual on the dirty side that the door is secure.
5. On the dirty side, close the ventilation valve (exhaust duct, #2) and the open the ball valve (atmospheric vent, #1).
6. Open the dirty side door to the pass-through. Remove the materials.
7. Disinfect the chamber and close the dirty side door.

DISINFECTANT DUNK TANKS

The chemical decontamination of material allows for some heat-sensitive equipment or material to be removed from the containment area. Chemicals, reagents or some tools will not withstand the heat and pressure that is used in autoclaves without breaking down. The dunk tank is used to decontaminate the outside surface of the containers that hold the material mentioned above. The use of the dunk tank requires the approval of the Biosafety Officer, and usually requires at least two people. **It may require other approvals to move live agents out of containment.**

From the Dirty Side

1. Fill out the log book indicating the:
    a. Date,
    b. Disinfectant in the tank,
    c. Material to be disinfected,
    d. Type of packaging,
    e. Person responsible for the material,
    f. The person placing the material into the tank, and
    g. The time the material entered the tank.
2. Unclip the locks and lift the door of the tank to open.
3. Place the material into the tank, making sure that it is pushed through to the other side (below the baffle), using the support rod. Air tight containers will float, so make sure that the basket is in place on the other side. Large objects will have to be checked to make sure that they do not displace more liquid than is required to maintain the airlock.
4. Close the door to the tank and replace the clips.
5. Contact the person who will be receiving the material on the clean side of the tank (this can be the Biosafety Officer (BSO), a staff member of the section, or the glass-wash person) and make arrangements for the time that is required. This should be arranged in advance. The minimum contact time is very important and depends on the decontaminant and its concentration. It may vary from 10 minutes to half an hour. This will be posted on both sides of the tank when the tank is filled.

From the Clean Side

No one should take anything out of the Dunk Tank unless they have been contacted by the BSO or the person who placed material into the tank. In order, to take biological (the BSO) or chemical waste (Chemical Safety Officer) out of containment other forms must be filled out in advance.

1. Open the door to the tank.
2. Lift the basket and let it drain. If the container has trapped any decontaminant, make sure it is emptied before removing.
3. Remove the material and allow it to dry off on the stainless steel cart.
4. Fill in the log including the :
    a. Date,
    b. Disinfectant,
    c. Material,
    d. Time and
    e. Your name.
5. Replace the basket.
6. Close the door to the tank.
7. Take the cart to the wash up area where it can be rinsed off.

MAINTENANCE

The level of disinfectant in the dunk tank is critical. The level of disinfectant must never be allowed to reach the barrier in the tank since this will cause a breach in the biocontainment barrier of the suite. Levels on the dunk tanks should be monitored on a daily basis and the level increased when they reach 3 cm below original full level. Some disinfectants can be replenished and some must be replaced. This can be determined by the manufacturer's product information. When you are checking the fill level it is necessary to check the condition of the gaskets on the doors.

Changing the Disinfectant

Changing the disinfectant is the operator's responsibility. The following sequence should be followed.

1. Seal and secure the access door on the clean side of the dunk tank. The door should be taped and labelled "Do Not Open" to avoid inadvertent opening while the tank is empty.
2. A hose should be attached to the drain on the dirty side of the dunk tank. Some tanks are direct gravity and some are pump assisted. The hose should be placed in a drain or a receptacle for disinfectant disposal later.
3. Open the drain valve and empty the tank. If the tank is pump assisted, do not turn on the pump until the drain valve is open.
4. When the contents of the tank are drained, rinse the tank with about 10 liters of water.
5. Turn off the pump and close the drain valve. Check the condition of the epoxy coating on the inside of the tank. If this coating is damaged, notify the Biosafety Officer.
6. Replenish the disinfectant in the tank.

FORMALDEHYDE DECONTAMINATION

The chief use of formaldehyde at Federal Laboratories is for DECONTAMINATING such things as laboratory equipment, light bulbs, computers - in fact, just about any clean item that needs to be taken out of the Biocontainment area but which cannot be decontaminated by steam sterilization or by immersing in the

dunk tank. It is also used for decontamination of room spaces such as animal rooms and air locks.

IT IS ESSENTIAL THAT AT LEAST TWO PERSONNEL, FAMILIAR WITH DECONTAMINATION WITH FORMALDEHYDE, BE AVAILABLE DURING ANY DECONTAMINATION PROCEDURE, AND SHOULD BE SUPERVISED BY A QUALIFIED REPRESENTATIVE OF SAFETY AND ENVIRONMENTAL SERVICES.

DECONTAMINATING BIOLOGICAL SAFETY CABINETS (BSC)

RECOGNIZE THAT IT IS VERY DIFFICULT TO ACHIEVE A 100% SEAL ON BIOLOGICAL SAFETY CABINETS - PARTICULARLY OLDER ONES. THEREFORE, ONLY PERSONNEL INVOLVED IN THE DECONTAMINATION SHOULD BE PERMITTED IN THE AREA WHERE THE DECONTAMINATION IS IN PROGRESS.

Equipment

- CERTEK Model # 1414RH Formaldehyde generator/neutralizer
- Paraformaldehyde, flake or prills are preferred, but powder may be used
- Ammonium carbonate
- Clear plastic tubing, 3/8 I.D. and tubing clamps
- Preformed "Blank-off" plates or clear polyethylene sheeting, 6 mil minimum thickness
- Duct tape
- De-ionized water is preferred. Tap water may be used
- Hygrometer and thermometer
- Respirator for formaldehyde. Must be properly fitted for personnel.
- Rubber gloves
- Formaldehyde detector tubes, such as Drager #QS-5462, and pump

Preparation

1. Determine the size of the space to be decontaminated by measuring the height, width, and depth in feet. Multiply the height by the width by the depth to determine the volume of the enclosure in cubic feet. For purposes of this calculation, items inside the space to be decontaminated are considered not to occupy any space (See Appendix I for BSC volumes).
2. Place a thermometer and hygrometer inside the enclosure **and** determine the temperature in degrees Fahrenheit and the relative humidity. Be sure that enough time is allowed for these gauges to stabilize so that an accurate reading may be determined.
3. The temperature should remain between 16 and 32 degrees C. for the best results. Relative humidity must be held between 50 and 90%. If the relative humidity is less than 60%, it must be increased by boiling water into the enclosure from the water canister on the generator. Add approximately 10 cc of water to the calculated amount, for water that will remain in the tubing and canister. Place biological indicators into the enclosure at this time.
4. Seal the enclosure with the blank-off plates and attach blower hoses. Operate the blower to determine if you have unrestricted airflow.
5. Determine the volume of paraformaldehyde required by multiplying the cubic feet of space to be decontaminated by .3 grams.
6. After determining the amount of paraformaldehyde required, the quantity of ammonium carbonate needed to neutralize this paraformaldehyde can be found. Multiply the grams of paraformaldehyde by the factor of 1.1 to determine the quantity of ammonium carbonate required. (Ammonium carbonate is very hygroscopic. The factor above is based upon the use of

fresh material. Should the ammonium carbonate be old, the factor will have to be increased to allow for the moisture that the ammonium carbonate has absorbed. Excessive ammonium carbonate simply causes an ammonia smell at the end of the process).

Setup

1. Place rear of generator as close to the formaldehyde insertion point as possible. Open the rear compartment of the cover, exposing the canisters.
2. Connect tubing from the three canisters (FOR-M, NEUT, and WATER, if required) to access openings in the space. (Of course, it is not necessary to connect tubing to the "WATER' canister if no water is to be added and the Bypass feature is to be used.) Also, connect tubing from the space for return air to the generator. This port is the one with the filter in the glass bottle adjacent to the formaldehyde canister. It is recommended that the tubing from the canisters be as short as possible and that the return-air tubing be the long one. Make sure that the tubing is replaced periodically and that they are not plugged.

   ALWAYS SECURE TUBING TO PREVENT IT FROM DISCONNECTING FROM THE INJECTION POINTS ON THE BLOWER HOUSING.
3. Remove the lid from the canister marked "FORM". Place the amount of paraformaldehyde that was determined to be sufficient in Step #5 above into the canister. Reinstall the lid.
4. Remove the lid from the canister marked "NEUT". Place the quantity of ammonium carbonate that was determined in Step #6 above in this canister. Rubber gloves should also be worn when weighing and handling the ammonium carbonate. Ammonia gas will build up inside the container of ammonium carbonate; therefore, care should be used in handling the ammonium carbonate to avoid breathing this concentrated gas. A chemical fume hood should be used to weigh the chemicals.
5. If water is required as determined in Step #3 above, add the water to the canister marked "WATER". Remember to add an additional 10 cc's to allow for water that will remain in the tubing and canister. Set the "Humidify/Bypass" switch to the Humidity position. If it is not necessary to add water, set the "humidify/Bypass" switch to the Bypass position.

   CAUTION!! DO NOT FORCE LID ONTO THE 'WATER' CANISTER - MAKE SURE THAT IT SLIPS ON EASILY. IT IS DESIGNED TO SLIP OFF EASILY IN THE EVENT OF A BLOCKAGE IN THE LINE.

   If the lid is not properly placed on the canister, the 'Lid Open" light will illuminate.

   Should the 'Lid Open" light be activated during the humidity insert cycle, the process will stop to allow the laboratory staff to determine the cause. The process will have to be re-initiated. DO NOT ATTEMPT TO RESTART IN THE MIDDLE OF THE CYCLE.
6. Set required **"**CONTACT TIME" on the Timer.
7. Plug the electrical cord of the Formaldehyde Generator into a 115v single-phase 60-cycle power supply**.**
8. Turn "POWER" switch to the "ON" position. This should cause the "Power On" and "Power Loss" lights to energize. The "Power Loss" light indicates that the generator has experienced a "Power Loss" since completing the previous cycle.
9. Push the button marked "RESET". This programs the generator to begin the next cycle. Please note that the "Sequence Complete" light is now activated, indicating that the previous cycle was successfully completed. If this light is not energized, the sequence timer did not reach its zero position from the previous cycle. See "Sequence Indicator Lights" section of these instructions for

an explanation of the various cycles and indicators, and the procedures to be followed to correct this situation.

DURING THE VARIOUS HEATING CYCLES THAT FOLLOW THE CANISTERS BECOME VERY HOT. DO NOT TOUCH THE CANISTERS UNTIL THEY HAVE HAD SUFFICIENT TIME TO COOL.

10. Push the "Start" button. This activates the "Auto Sequence" light and one or two of the following: (a) If the "Humidify/Bypass" switch is in the humidify position, the heater on the "WATER" canister begins to heat the water to boil it off. - or (b) If the "Humidify/Bypass" switch is in the bypass position, the formaldehyde insert cycle begins. The "Form Insertion" light is energized, the pump begins, and "FORM" canister heater is activated. The Formaldehyde Insert Cycle automatically begins after all the water is boiled off if the "Humidify/Bypass" switch is placed in the "Humidify" position.
11. It takes approximately 15-30 minutes to boil off the water, depending upon how much water must be vaporized.
12. The "Formaldehyde Insert" cycle operates for 50-55 minutes. After this cycle is complete, the blower and formaldehyde heater are deactivated. The "Contact Timer" is then activated and controls the instrument until the selected "Contact Time" is timed out.
13. At the end of the "Contact Time", the "Neutralizer" canister heater, blower and solenoid valves are activated and the neutralization insertion begins. The "Neut Insert" light will be activated. This cycle requires 50-55 minutes.
14. At the completion of the neutralizer insert cycle, the "Neut Contact" light is activated and this portion of the cycle begins. There is a built-in hold time of 50-55 minutes for this chemical reaction to occur. If there was a small amount of formaldehyde and neutralizer used, this cycle may be shortened by advancing the control timer at the rear of the unit. Caution must be used to ensure that enough time is allowed for the formaldehyde to be completely reacted with the ammonium carbonate.
15. At the end of the "Neut Contact" cycle, the instrument goes into the 'Form Insert" cycle for approximately 1 minute. This prepares the instrument for the next cycle.
16. If the cycle was completed successfully as programmed, the "Sequence Complete" light will then be energized. The unit will remain in this configuration until the power switch is moved to "Off".
17. At the end of the cycle, the following items need to be noted, and for laboratory safety, recorded on a permanent record:
    a. Date and Time
    b. Equipment or space decontaminated
    c. Weight of Paraformaldehyde and ammonium carbonate used
    d. The contact time as recorded on the timer
    e. The lot number of the spore strip or other biological indicator if one was used.
18. Prior to opening the space, open the formaldehyde canister and Neutralization canister to ensure that both were depolymerized. Carefully open the space, making sure that complete neutralization occurred.
19. At the end of the cycle, the product of the neutralization, hexamethylene tetramine, a white powder may be visible. This may be cleaned off with a rag dampened with ethanol or left for approximately 8 hours and it will also depolymerize from the surface.

HEPA HOUSINGS

Prior to breaching the security of the HEPA housing, it is required that they be decontaminated to protect the technician from exposure to etiologic agents and to prevent the release of diseases to the environment. This is done prior to HEPA change, HEPA certification and repairs to housings or dampers.

Equipment

- CERTEK Model # 1414RH Formaldehyde Generator/Neutralize
- Decontamination cart with 5 hp blower
- Sections of 3in decon hosing
- Formaldehyde warning tape and signs
- Biological indicators
- Paraformaldehyde, flake or prills are preferred, but powder may be used
- Ammonium carbonate
- Clear plastic tubing, 3/8 I.D. and tubing clamps
- Duct tape
- Deionized water - preferred. Tap may be used
- Hygrometer and thermometer
- Respirator for formaldehyde. Must be properly fitted for personnel
- Rubber gloves
- Formaldehyde detector tubes, such as Drager #QS-5462, and pump

Preparation

During the decontamination, it should NEVER be assumed that the bioseal dampers will contain the formaldehyde gas in the housing. Therefore, notification of laboratory staff and restricted access to the areas affected is required until the decontamination is complete.

1. Determine the size of the space to be decontaminated by measuring the height, width, and depth in feet. Multiply the height by the width by the depth to determine the volume of the enclosure in cubic feet. For purposes of this calculation, items inside the space to be decontaminated are considered not to occupy any space.
2. Determine the temperature in degrees Fahrenheit and the relative humidity at the balancers sampling port, located just prior to the exhaust box.
3. The temperature should remain between 16 and 32 degrees C. for the best results. Relative humidity must be held between 50 and 90%. If the relative humidity is less than 60%, it must be increased by boiling water into the enclosure from the water canister on the generator. Refer to the graph or Psychrometric Chart Method near the end of these instructions to determine the amount of water required. Add approximately 10 cc of water to the calculated amount.
4. Determine the volume of paraformaldehyde required by multiplying the cubic feet of space to be decontaminated by .3 grams.
5. After determining the amount of paraformaldehyde required, the quantity of ammonium carbonate needed to neutralize this paraformaldehyde can be found. Multiply the grams of paraformaldehyde by the factor of 1.1 to determine the quantity of ammonium carbonate required. (Ammonium carbonate is very hygroscopic. The factor above is based upon the use of fresh material. Should the ammonium carbonate be old the factor will have to be increased to allot for the moisture that the ammonium

carbonate has absorbed. Excessive ammonium carbonate simply causes an ammonia smell at the end of the process.)

Setup

1. Place the rear of Generator and decon cart as close to the formaldehyde insertion point as possible. Open the rear compartment of the cover, exposing the canisters.
2. Connect tubing from the three canisters (FOR-M, NEUT, and WATER, if required) to access openings in the fan housing. (Of course, it is not necessary to connect tubing to the "WATER' canister if no water is to be added and the Bypass feature is to be used.) Also, connect tubing from the space for return air to the generator. This port is the one with the filter in the glass bottle adjacent to the formaldehyde canister. It is recommended that the tubing from the canisters be as short as possible and that the return-air tubing be the long one. Make sure that the tubing and ports are replaced periodically and that they are not plugged.
3. Seal the housing by notifying the control tech to shut down the fan for the specific area, then the automatic damper should be overridden at the housing to insure that it cannot be accidentally opened at the EMCS during the decontamination process. Then close the manual damper and attach blower hoses. Supply side of air should follow normal flow and be upstream and the return hose should be down-stream. A biological indicator should be placed in downstream hose connection. Open the butterfly valves and operate the blower to determine if you have unrestricted airflow.

ALWAYS SECURE TUBING TO PREVENT IT FROM DISCONNECTING FROM THE INJECTION POINTS ON THE BLOWER HOUSING.

4. Remove the lid from the canister marked "FORM". Place the amount of paraformaldehyde that was determined to be sufficient in Step #5 above into the canister. Reinstall the lid.
5. Remove the lid from the canister marked "NEUT". Place the quantity of ammonium carbonate that was determined in Step #6 above. in this canister. Rubber gloves should also be worn when weighing and handling the ammonium carbonate. Ammonia gas will build up inside the container of ammonium carbonate; therefore, care should be used in handling the ammonium carbonate to avoid breathing this concentrated gas. A chemical fume hood should be used when weighing the chemicals.
6. If water is required as determined in Step #3 above, add the water to the canister marked "WATER". Remember to add an additional 10 cc's to allow for water that will remain in the tubing and canister. Set the "Humidify/Bypass" switch to the Humidity position. if it is not necessary to add water, set the "humidify/Bypass" switch to the Bypass position.

CAUTION!! DO NOT FORCE LID ONTO THE 'WATER' CANISTER - MAKE SURE THAT IT SLIPS ON EASILY. IT IS DESIGNED TO SLIP OFF EASILY IN THE EVENT OF A BLOCKAGE IN THE LINE.

If the lid is not properly placed on the canister, the "Lid Open" light will illuminate.

Should the 'Lid Open" light be activated during the humidity insert cycle, the process will stop to allow for the determination of what the cause was. The process will have to be reinitiate. DO NOT ATTEMPT TO RESTART IN THE MIDDLE OF THE CYCLE.

7. Set required **"**CONTACT TIME" on the timer.
8. Plug the electrical cord of the Formaldehyde Generator into a 115v single-phase 60-cycle power supply.
9. Turn "POWER" switch to the "ON" position. This should cause the "Power On" and "Power Loss" lights to energize. The "Power Loss" light indicates that the Generator has experienced a "Power Loss" since completing the previous cycle.

10. Push the button marked "RESET". This programs the generator to begin the next cycle. Please note that the "Sequence Complete" light is now activated, indicating that the previous cycle was successfully completed. If this light is not energized, the sequence timer did not reach its' zero position from the previous cycle. See "Sequence Indicator Lights" section of these instructions for an explanation of the various cycles and indicators, and the procedures to be followed to correct this situation.

DURING THE VARIOUS HEATING CYCLES THAT FOLLOW THE CANISTERS BECOME VERY HOT. THEREFORE, MAKE SURE THAT YOU NOT TOUCH THE CANISTERS UNTIL THEY HAVE HAD SUFFICIENT TIME TO COOL **DOWN.**

11. Activate the blower motor. Push the "Start" button. This activates the "Auto Sequence" light and one or two of the following: (a) If the "Humidify/Bypass" switch is in the Humidify position, the heater on the "WATER" canister begins to heat the water to boil it off. – or (b) If the "Humidify/Bypass" switch is in the Bypass position, the Formaldehyde Insert cycle begins. The "Form Insertion" light is energized, the pump begins, and "FORM" canister heater is activated. The Formaldehyde Insert Cycle automatically begins after all the water is boiled off if the "Humidify/Bypass" switch is placed in the "Humidify" position.
12. Boiling off the water takes approximately 15-30 minutes depending upon how much water must be vaporized.
13. The "Formaldehyde Insert" cycle operates for 50-55 minutes. After this cycle is complete, the blower and formaldehyde heater are deactivated. The "Contact Timer" is then activated and controls the instrument until the selected "Contact Time" is timed out.
14. At the end of the "Contact Time", the "Neutralizer" canister heater, blower and solenoid valves are activated and the neutralization insertion begins. The "Neut Insert" light will be activated. This cycle requires 50-55 minutes.
15. At the completion of the neutralizer insert cycle, the "Neut Contact" light is activated and this portion of the cycle begins. There is a built in hold time of 50-55 minutes for this chemical reaction to occur. If there was a small amount of formaldehyde and neutralizer used, this cycle may be shortened by advancing the control timer at the rear of the unit. Caution must be used here to ensure that enough time is allowed for the formaldehyde to be completely reacted with the ammonium carbonate.
16. At the end of the "Neut Contact" cycle, the instrument goes into the 'Form Insert" cycle for approximately 1 minute. This prepares the instrument for the next cycle.
17. If the cycle was completed successfully as programmed, the "Sequence Complete' light will then be energized. The unit will remain in this configuration until the power switch is moved to "Off".
18. At the end of the cycle the following need to be noted, and for laboratory safety, recorded on a permanent record:
    a. Date and Time
    b. Equipment or space decontaminated
    c. Weight of Paraformaldehyde and ammonium carbonate used
    d. The contact time as recorded on the timer
    e. The lot number of the spore strip or other biological indicator if one was used.
19. Prior to opening the space, open the Formaldehyde canister and Neutralization canister to ensure that both were depolymerized. Close the butterfly valve at the return end of housing and remove the biological indicator. **Do Not open the housing until the results of the biological indicator are received.** Carefully open the space, making sure that complete neutralization occurred. **Respiratory protection should be worn at this time.**

20. At the end of the cycle, the product of the neutralization, hexamethylene tetramine, a white powder may be visible. This may be cleaned off with a rag dampened with ethanol or left for approximately 8 hours and it will also depolymerize from the surface.

AIR LOCKS, ANIMAL CUBICLES, AND LABORATORIES

Equipment

The equipment necessary for the formaldehyde decontamination of air locks, animal cubicles and laboratories is all the same, i.e.:

- Electric frying pans capable of 400 °F
- Paraformaldehyde, flakes or prills are preferred, but powder may be used
- Ammonium carbonate
- Extension cords, two types needed: 1) twist-loc male end with standard female end; 2) standard male and female ends
- Deionized water-preferred. Tap may be used
- Hygrometer and thermometer
- Full-face respirator with cartridges suitable for formaldehyde. Must be properly fitted for personnel
- Rubber gloves
- Formaldehyde Detector Tubes, such as Dräger #QS-5462, and pump
- Duct tape
- Polyethylene vapor barrier, 6 mil
- *Biosign* Biological Indicators, and incubator (37°C)
- Formaldehyde Warning Tape and Signs

Preparation

IT IS ESSENTIAL THAT AT LEAST TWO PERSONNEL, FAMILIAR WITH DECONTAMINATION WITH FORMALDEHYDE, BE AVAILABLE DURING ANY DECONTAMINATION PROCEDURE, AND SHOULD BE SUPERVISED BY A QUALIFIED REPRESENTATIVE OF SAFETY AND ENVIRONMENTAL SERVICES.

During these decontaminations it should NEVER be assumed that the bioseal doors or poly protection will contain the formaldehyde gas in the area being decontaminated. A buffer zone surrounding the area should be established with Formaldehyde Tape and access controlled.

NOTE: For room decontaminations with formaldehyde gas, all room and biological safety cabinet bioseal dampers must be completely closed and the room must be completely sealed to render the room air-tight, i.e. if the room has submarine doors they should remain closed through entire procedure and if the area is not equipped with bioseal doors (H-block BSL-3), all perimeter doors must be sealed with poly and duct tape.

Formaldehyde gas has specific limitations. It will not effectively decontaminate porous or organic materials (i.e., paper, cardboard, cloth, Styrofoam, etc.) materials and these should be cleared from the area and decontaminated in some other way. Also, Ammonium Carbonate will not effectively neutralize any formaldehyde gas which penetrates these materials and will cause an exposure problem when handled.

1. Determine the size of the room to be decontaminated by measuring the height, width and depth in feet. Multiply the height by the width by the depth to determine the volume of the room in cubic feet. For the purpose of this calculation, items inside the room are not considered to occupy any space

2. Determine the volume of paraformaldehyde required by multiplying the cubic feet of the space to be decontaminated by 0.3 grams.
3. The quantity of ammonium carbonate needed to neutralize this amount of paraformaldehyde can be calculated by multiplying the grams of paraformaldehyde by the factor of 1.1. (NOTE: Ammonium carbonate is very hygroscopic. The factor above is based on the use of fresh chemical. For older ammonium carbonate, the factor will have to be increased to allow for the moisture that the chemical has absorbed. Excessive ammonium carbonate causes an ammonia smell at the end of the process.)
4. Weigh out predetermined amount of chemicals into *Zip-lock* plastic containers (946ml), or other suitable container on a top-loading laboratory balance located inside a fumehood.
5. Determine the temperature and humidity inside of the room by taking a reading with *Velocicalc Portable Air Velocity Meter,* using the temperature and humidity settings, on the clean side of the exhaust duct (i.e. the clean side of the bioseal damper). The temperature should be 21.1EC (70EF) or higher, and humidity should be 60 to 85%. If the relative humidity is < 60% it must be increased by adding a predetermined amount of water to the pan containing the paraformaldehyde.

Setup

1. Place electric frying pans around room being decontaminated near to outlets using extension cords. Two frying pans are needed at each location, one for the paraformaldehyde and one for the ammonium carbonate.
2. NOTE: In rooms where decon can be done remotely (e.g. HC BSL-4 lab), the outlets (red) are marked decon only (one twist-loc receptacle and one standard receptacle at each point). In this case, the parafarmaldehyde pan is connnected to an extension cord with a twist-loc male plug into the special receptacle. The ammonium carbonate is attached to a regular 3-prong extension cord and into the receptacle next to the one for paraformaldehyde.
3. Duct tape temperature setting dial to maximum on the pan; plug in pan in lab to test before filling with chemical.
4. Fill electric frying pans with appropriate chemical. Add predetermined amount of water to pan containing paraformaldehyde if the relative humidity in the room is < 60%.
5. NOTE: If the electric frying pans are being filled in a BSL-3 area, full-face respiratory protection is needed. A positive-pressure suit will be used in BSL-4.
6. Place a number of biological indicators around the room.
7. or barrier protected containment areas (e.g. HC BSL-4): Leave laboratory. Start decon via remote buttons on outside of lab. Timing sequence is 2 1/2 hours burn off for paraformaldehyde, 4 hours contact time followed by 2 1/2 hours burn off for ammonium carbonate, then 4 hours contact time.
8. For containment areas not protected with bioseal doors and sperate decontamination circuitry: Electric frying pans containing paraformaldehyde and ammonium carbonate should be hooked up to 20 amp separate electrical circuits so that they can be turned on from outside of the containment suite at the required times. The timing sequence is the same as noted above. Before sequence is started, all perimeter doors must be sealed with poly and duct tape to be air tight.
9. After complete sequence is timed out, air system must be re-started. Ventilation system should be run at least 24 hours prior to monitoring for residual gases.
10. Using a full face respirator, collect all biological indicators, then incubate at 37°C for 48 hours (the room is not considered 'clean' until there is a negative result for all biological indicators).
11. Using a full-face respirator, enter room and take an air quality reading with the Dräger Formaldehyde Detector system with activator tube. If there is less than .04 ppm formaldehyde detected, personnel may enter the area without face protection.

12. If there is a residue left on the surfaces in the lab, full-face respirators, gloves and cover-alls are to be worn to clean the lab (use an ammonia-based cleaning compound). This residue occurs if there is repolymerization of the paraformaldehyde on the lab surfaces.