JOHNS HOPKINS INSTITUTIONS

BIOSAFETY MANUAL

June 1995

To obtain additional copies of this manual call the Office of Safety and Environmental Health (OSEH), (410) 955-5918.

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FOREWARD

The Johns Hopkins Hospital and the Johns Hopkins University have high standards in teaching and research. The University and Hospital are committed to provide an academic and working environment as free as possible from all hazards. If we are to honor our commitment we must have the continued cooperation of faculty, administration, research and supervisory personnel for the establishment of safe procedures and for providing good training and adequate facilities for the protection of employees, students, patients and visitors.

The administration of the safety program has been placed with the Office of Safety and Environmental Health which offers a wide range of technical assistance, training aids and reference materials in biohazard control. Please use these resources to implement your safety program.

Edward J. Bernacki, M.D., M.P.H., Chair
Joint Committee on Health, Safety and Environment

PREFACE

This Biosafety Manual was prepared to provide laboratory and clinical investigators current information on biohazard control. The manual will be updated as our knowledge of the risks involved in the handling of biological hazards increases and as methods for containment of these hazards improve.

The Biosafety Manual brings together information which will assist supervisors in carrying out their responsibilities in the management of biohazards. The Manual provides suggested procedures, techniques and equipment to protect personnel, animals and experiments from unintentional infection or contamination in addition to pertinent policy generated by Hopkins committees and standards resulting from federal, state and local legislation.

The manual represents a compendium of biohazards control information that can serve as a base for specific programs. All personnel using potentially biohazardous materials should become familiar with the Biosafety Manual and should conduct their operation in accordance with the level of risk appropriate for the procedure and the potentially biohazardous agent or materials they handle.

The success of the biohazardous control program depends on investigators who are motivated toward a safe working environment and who have knowledge of safe operational procedures. The Biosafety Office will endeavor to do its part by providing accurate information and technical assistance to assist in the establishment of adequate biohazards controls for the protection of both the investigators and their experiments.

Richard W. Gilpin, Ph.D., R.B.P.
Biosafety Officer

Byron S. Tepper, Ph.D., C.S.P.
Director, Office of Safety & Environmental Health

TABLE OF CONTENTS

PAGE

SECTION I -- SAFETY RESPONSIBILITIES

Administrative Responsibilities 1
Administrative Officers 1
Department Chairman and/or Director 1
Principal Investigator and/or Supervisor 1
Individual Researchers and/or Technicians 2
Periodic Reviews and Updates of Registered Research 3
Biological Agents and/or Materials 3
Recombinant DNA 3
Registration of Research with
Pathogenic and/or Oncogenic Materials 4
Background 4
Definition 4
Responsibility 4
Assessment of Hazard 5
Purpose of Registration 5
Procedure for Registration 6
Standard Operating Procedure (SOP) Manual
for Biosafety Level 3 Laboratories 7
Vaccinia Virus Vaccination 8
Registration of Research with HBV and/or HIV 9
Requirements 9
Informed Consent for Use of Postexposure Zidovudine (AZT) 9
Registration of Research with Sheep or Goats 12
Background 12
Recommended Control Measures for Q Fever 12
Registration of Research with Recombinant DNA 14
Registration Form 14
Review Procedure 14
Review of Recombinant DNA Research With Moderate Risk
Biological Agents or Materials at Biosafety Level 1 or 2 15
Review of Recombinant DNA Research Involving Biosafety Level 3
for High Risk Biological Agents or Materials or Recombinant
DNA Involving Human Subjects 15
Registration of Research with Non-Human Primates 17
Tuberculosis 17
Herpesvirus-B in Macaque Monkeys 18

TABLE OF CONTENTS

PAGE

SECTION II -- SAFETY COMPLIANCE
Standard Practices, Training and Precautions 21
Universal Precautions Guidelines for Research Laboratories 23
  Definitions 23
  Precautions 23
HBV and/or HIV Agent Summary Statements 27
Q Fever Precautions 28
  Background 28
  Rationale 28
  Registration 28
  Medical Surveillance 29
  Transport of Animals 29
  Personnel Protection 30
  Waste Disposal 31
  Decontamination 31
Biosafety Level 1 Guidelines 32
Biosafety Level 2 Guidelines 33
Biosafety Level 3 Guidelines 34
Animal Biosafety Levels 35
Biosafety References 36
Biosafety Level 3 Facility Specifications 38
  Performance 38
  Location 38
  Floors 38
  Walls 38
  Ceiling 38
  Access Zone 38
  Doors 38
  Windows 39
  Plumbing 39
  Vacuum System 39
  Electrical 39
  Light Fixtures 39
  Lab Furniture 39
  Hand Washing Sink 40
  Eye, Face and Body Spray Fixture 40
  Autoclave 40
  Ventilation 40
  Biological Safety Cabinets 41
  Chemical Fume Hood 41
  Air Pressure Differential 41
  Directional Airflow 42
  Air Supply Vents 42

TABLE OF CONTENTS

PAGE

Fire Protection 42
Summary of Biosafety Levels 43
Classification of Etiologic Agents on the Basis of Hazard 44
Classification of Ongogenic Viruses on the Basis of Hazard 45
Criteria for Moderate Risk Oncogenic Viruses 45
Criteria for High Risk Oncogenic Viruses 45
General Laboratory Practices and Procedures 46
Employee Action 46
Food, Drink, and Cosmetics 46
Hand Washing 46
Beards and Long Hair 47
Books and Journals 47
Pipettes 47
Syringes 48
Disposal of Research Laboratory Waste 49
Definition of Sharps 49
Disposal of Laboratory Waste 49
Approved Laboratory Waste Containers 49
Utilization of Six or Ten Quart Plastic Containers 50
Utilization of the Biohazard Box 50
Additional Containers 51
Safe Disposal of Sharps Containers 51
Disposal of General Laboratory Glass 52
Summary of Waste Disposed 52
Centrifuge Containment 53
Centrifuge Precautions 53
Centrifuge Tubes 53
High-Speed Centrifuges 54
Containment Centrifugation 54
Blenders, Sonicators, Mills, Grinders & Cell Sorters 57
Water Bath Disinfectants 58
Laboratory Vacuum Traps 59
Refrigerators, Freezers and Dry Ice Chests 61
Transportation of Biohazardous Material 62
Test Tube and/or Vacutainer Techniques 63
Membrane Filters 64
Hazardous Operations (Two Person Rule) 65
Use of Dry Powders 66
Laboratory Construction and Renovation 67
Animal Care and Handling 68
Decontamination and Disinfection Procedures 69

TABLE OF CONTENTS

PAGE

Steam Autoclaving 71
Autoclave Decontamination Procedures 72
  Background 72
  Indicators 72
  Containers 74
  Autoclave Operation 75
  Processing Times 76
  Odor Control 77
  Training 77
  Record Keeping 77
  Autoclave Log Book Samples 78
Dry Heat Sterilization 83
Ethylene Oxide Sterilization 84
Formaldehyde Gas Decontamination of Equipment 85
Ultraviolet Light Decontamination 86
  UV Lamp Operation 86
  Training 87
Chemical Disinfectants 88
  Phenolic Compounds 88
  Quaternary Ammonium Compounds 88
  Iodophors 89
  Alcohols 90
  Aldehydes 90
  Chlorine Compounds 91
  Mercurials 91
Clean-up of Biohazardous Spills 93
  Biohazard Spill Inside a Biological Safety Cabinet 93
  Biohazard Spill Outside a Biological Safety Cabinet 93
  Radioactive Biohazard Spill Outside a Biological Safety Cabinet 95
Shipment of Biological Materials 97

SECTION III -- RECOMBINANT DNA GUIDELINES

  Introduction 101
  Table of Contents 101
  Section I. Scope of the NIH Guidelines 104
  Section II. Containment 106
  Section III. Experiments Covered by the NIH Guidelines 107
  Section IV. Roles and Responsibilities 114
  Section V. Footnotes and References of Sections I Through IV 124
  Appendix A. Exemptions Under Section III-E-5
    Sublists of Natural Exchangers 126

TABLE OF CONTENTS

PAGE

Appendix B. Classification of Etiologic Agents and Oncogenic Viruses on the Basis of Hazard 127
Appendix C. Exemptions Under Section III-E-6 133
Appendix D. Major Actions Taken Under the NIH Guidelines 136
Appendix E. Certified Host-Vector Systems 136
Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates 138
Appendix G. Physical Containment 139
Appendix H. Shipment 151
Appendix I. Biological Containment 152
Appendix J. Biotechnology Research Subcommittee 155
Appendix K. Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules 155
Appendix L. Release into the Environment of Certain Plants 165
Appendix M. Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into the Genome of One or More Human Subjects 165
Appendix P. Physical and Biological Containment for Recombinant DNA Research Involving Plants 176
Appendix Q. Physical and Biological Containment for Recombinant DNA Research Involving Animals 187

SECTION IV -- BIOHAZARD CABINETRY

Biohazard Containment Equipment 202
Types of Containment Equipment 202
Use of Containment Equipment 202
Biohazard Cabinet Containment Equipment 203
Biological Safety Cabinet Categories 205
Use of Biological Safety Cabinets 208
Operation of Biological Safety Cabinets 210
General Suggestions 210
Starting Up Your BSC 210
Cleaning Your BSC 213
Flammable or Explosive Materials within BSC's 214
BSC Assignment for Specific Biosafety Levels 215
Biosafety Cabinet Training Materials 216
Characteristics of BSC'S and other Airflow Equipment 218
Class 1 Biological Safety Cabinets 219
Class II, Type A Biological Safety Cabinets 221
Class II, Type B1 Biological Safety Cabinets 223

TABLE OF CONTENTS

PAGE

Class II, Type B2 Biological Safety Cabinet 226
Class II, Type B3 Biological Safety Cabinets 228
Class III Biological Safety Cabinets 230
Other Cabinets 232
Horizontal Laminar Flow Clean Air Benches (CAB's) 232
Chemical Fume Hoods 234
Biological Safety Cabinet Use Guidelines 236
Purchasing Procedures for Biological Safety Cabinets 237
Vendors of Biological Safety Cabinets 242
Biological Safety Cabinet Installation Guidelines 244
Set-upLocation 244
Gas and Vacuum Utility Connections 246
Electrical Service Connections 246
New Installations 247
Working Height 247
Thimble Connection Exhaust Air Venting 248
Hard Connection Exhaust Air Venting 255
Frequency of Cabinet Certification 257
Annual Cabinet Labor and Service Contract 258
Scheduling Cabinet Service and Certification 259
Moving Your Cabinet 261
Certification Tests 262

SECTION V -- HAZARD WARNING SIGNAGE

Hazard Signage 265
Hazard Warning Labels 269
Caution Signage
Biohazard, Infectious Agents, BL2 278
Biohazard, Potentially Infectious Material, BL2 280
Biohazard, Infectious Agents, BL3 282
Biohazard, Infected Animals 284
Radioactive Materials 286
Radiation Area 288
High Radiation Area 290
Hazardous Chemical, Cancer Suspect Agent 292
Cancer Hazard 294
Eye Protection Required 296
Danger, Do Not Enter, Contaminated Area 298
Multiple Hazards 300

INDEX 303

ADMINISTRATIVE RESPONSIBILITIES

A. ADMINISTRATIVE OFFICERS

The Administrative Officers of the Johns Hopkins Institutions have the responsibility to ensure that all research and investigative activities under their control are conducted in a manner that presents the least possible hazard to employees, patients, students, visitors, and to the surrounding community.

B. DEPARTMENT CHAIRMAN and/or DIRECTOR

Responsibility for the health and safety of employees, visitors and students ultimately rests with each department head. They interpret institutional policies and recommendations and assure compliance with their provisions. Each department head is responsible for approving all research and all protocols for research involving hazardous biological agents prior to their initiation.

C. PRINCIPAL INVESTIGATOR and/or SUPERVISOR

Supervisors at every level are responsible for biohazard control. They are responsible for assuring appropriate training of employees on safe practices, for correcting errors and defective conditions which could result in personal injury and/or property damage, and for developing a positive attitude among employees toward biohazard safety and accident prevention.

Other specific biohazard control responsibilities of Principal Investigators and Supervisors are to:

1. Develop specific protocols to ensure safe practices and procedures in the use of biological agents.

2. Obtain appropriate approvals of protocols for biohazardous research from the Department Chairman and Institutional Biosafety Committee prior to the initiation of research.

3. Implement policies, procedures and methods approved by the Joint Committee on Health, Safety and Environment.

4. Properly register all potentially infectious agents or materials and recombinant DNA projects with the Biosafety Officer.

5. Properly register all Biological Safety Cabinets and Clean Air Benches with the Biosafety Officer and participate in the annual or semi-annual certification program.

D. INDIVIDUAL RESEARCHERS and/or TECHNICIANS

Each employee is responsible for complying with all safety rules, regulations, and procedures required for the task assigned. Each employee is responsible for reporting all incidents resulting in or having the potential for personal injury, illness and/or property damage or any action or existing condition which may result in such accidents.

PERIODIC REVIEWS AND UPDATES OF REGISTERED RESEARCH

The Biosafety Officer will make periodic inspections of all projects involved with moderate or high risk biological agents or materials (Biosafety Level 2 or higher) to assure continued compliance with biohazard control requirements. All recombinant DNA research programs covered by the NIH Guidelines will be inspected for adherence to the provisions of the NIH Guidelines.

A. BIOLOGICAL AGENTS AND/OR MATERIALS

Every project involving biological agents or materials, sheep, goats, non-human primates, and other potentially pathogenic materials will be reviewed annually by the Biosafety Officer.

B. RECOMBINANT DNA

All research involving recombinant DNA shall be updated annually by the Biosafety Officer.

REGISTRATION OF RESEARCH WITH PATHOGENIC AND/OR ONCOGENIC MATERIALS

It is the responsibility of each principal investigator or laboratory director, with the advice of the Biosafety Officer, to identify all biohazardous agents or materials in use or in stock and file a Potentially Pathogenic and/or Oncogenic Agent or Material Registration Form with the Office of Safety and Environmental Health. The Biosafety Officer should be notified whenever a new etiological agent or biohazardous material is identified or introduced into use.

A. BACKGROUND

From 1970 through 1982 the Johns Hopkins Institutions Joint Commission on the Use of Infectious Agents and Other Biohazardous Materials investigated the potential hazards associated with the use of biological agents and materials. A survey by the committee identified over 130 laboratories in which one or more infectious agents were used in investigative research. The committee recognized the necessity for updating and expanding the database of the initial survey and recommended the establishment of a central registry of biological agents and materials in use.

B. DEFINITION

For the purpose of this registry, pathogenic or oncogenic agents or materials shall include known human and animal pathogens and oncogenic viruses; potential or suspect etiologic agents; tissue cultures, tissues, blood and body fluids, specimens and samples presumed to contain etiologic agents; microbial toxins; and organisms involved in genetic manipulation experiments without consideration of the degree of infectivity, pathogenicity or public health risk. A degree of hazard or risk will be assigned to the registered agents or materials by the Biosafety Officer and/or the Institutional Biosafety Committee based on the information supplied by the investigator, current classifications of etiologic agents, and current publications.

C. RESPONSIBILITY

1. The Johns Hopkins Institutions Biosafety Officer, Office of Safety and Environmental Health, is responsible for the maintenance of the registry.

2. It is the responsibility of each principal investigator to furnish information for all biohazardous agents and materials presently in use in investigative research and for all agents maintained in stock culture collections for research or teaching purposes.

3. It is the responsibility of each principal investigator to provide this information whenever a new biological agent or material is identified or introduced into use.

D. ASSESSMENT OF HAZARD

The level of competence and physical containment required for work with biological agents or materials is dependent on an evaluation of the degree of hazard associated with each research protocol. It should be noted that the criteria for establishing levels are not absolute. The degree of hazard depends on the agent and the risk of exposure which may vary depending on the nature and kind of study in which the biological agent or material is used.

Aerosol studies, passage in animals, and infection of vectors are examples of procedures which markedly increase the hazard, whereas strict adherence to in vitro experiments or the use of attenuated agents decrease the hazard. The hazard/risk levels will be evaluated for each protocol to determine appropriate biohazard safety controls.

E. PURPOSE OF REGISTRATION

The purpose of completing the registration form is to ensure adequate review to determine compliance with the biohazards safety regulations regarding the safe handling, storage, and disposal of hazardous agents and to provide emergency information.

The registration form must be submitted when:

1. An investigator plans a laboratory project or procedure involving:

a. Biological agents requiring Biosafety Level 2, 3, 4, or 5 (Class 2, Class 3, Class 4 and Class 5).

b. Agents classified as low-risk, moderate-risk and high-risk oncogenic viruses.

c. Agents involved in research with recombinant DNA molecules.

d. Materials associated with potentially infectious bloodborne pathogens such as; all human and nonhuman primate blood, body fluids containing visible blood, tissue specimens, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, semen, vaginal secretions, and breast milk. This includes fresh human specimens removed at surgery and submitted to the Department of Pathology.

e. Tissue cultures, tissues, body fluids, specimens and samples presumed to contain etiologic or oncogenic agents.

2. The form may also be used when an investigator wants the Biosafety Officer to review safety and containment procedures, e.g.:

a. A new technique or procedure is to be used.

b. An existing technique is to be modified and the investigator believes that the modification may increase the risk to laboratory personnel or animal caretakers.

c. An antigen, whose risks are well understood by the investigator, is to be highly concentrated with possible increased risk.

d. When an infectious agent is to be used which makes vaccination of personnel desirable, prior to the onset of work.

e. When the containment equipment or laboratory facility is altered, moved, modified, replaced, or supplemented.

F. PROCEDURE FOR REGISTRATION

1. Preliminary Discussion
Contact the Biosafety Officer to determine if submission of a registration form will be necessary for the proposed work and, if so, obtain a copy from the Office of Safety and Environmental Health.

2. Submission of Form
Submit the completed form to the Biosafety Officer. The form should be submitted for committee review one (1) week prior to deadline. For new grants, in particular, it is to the advantage of the investigator to allow enough time to permit inclusion of the Institutional Biosafety Committee's action on the protocol with the grant application.

3. Processing of Form
When the Biosafety Officer receives the protocol, the agent will be classified according to guidelines and the requirement for further review will be determined. If necessary, the investigator and the Biosafety Officer or appropriate designee together will evaluate the project protocol, equipment, work space and other pertinent information.

The Biosafety Officer will present projects requiring Biosafety level 3 and above to the Institutional Biosafety Committee and/or the Joint Committee on Health, Safety and Environment, when applicable, and will write a report on their actions defining the conditions for project approval. A copy of the report will be sent to all personnel who need information about the work, (e.g.; the investigator, the department chairman / director, and the dean of the appropriate university division).

4. Protocols

The principal investigator or laboratory director in charge of all projects or procedures involving the use of moderate or high-risk biological agents or materials shall prepare a written protocol including, but not limited to, the following information to be submitted to

the Biosafety Officer along with the Potentially Pathogenic / Oncogenic Agent or Material Registration Form:

a. Identification of the etiologic or oncogenic agent or material (genus, species, type), pathogenicity of the agent, and host range if known.

b. Description of the experiment and techniques to be used and quantities of organisms to be used.

c. Techniques, facilities and methods to assure containment.

d. Method for terminal inactivation of the agent or material.

e. Special requirements for handling experimentally infected animals.

G. STANDARD OPERATING PROCEDURE (SOP) MANUAL FOR BIOSAFETY LEVEL 3 LABORATORIES

In addition to the items listed in "4" above, a Standard Operating Manual shall be prepared by the principal investigator or laboratory director and submitted to The Johns Hopkins Institutions Biosafety Officer for review. A hard copy and computer disk "template manual" are available from the Biosafety Officer. The pages of this manual shall be numbered consecutively and dated. The manual shall contain a:

1. "Table of Contents" page

2. List of personnel permitted to work in the laboratory

3. List of chemicals and materials kept in the laboratory

4. Floor plan of the laboratory

5. List of emergency contact numbers and procedures to be followed by personnel during an emergency

6. Discussion of operating hours and procedures for admittance of housekeeping or maintenance personnel for routine or special functions

7. Discussion of decontamination and waste disposal procedures to be followed by laboratory personnel, including autoclave guidelines and log sheets.

8. Discussion of the types, locations and uses of available personnel protective equipment

9. Specific experimental procedures (SOP's) to be followed in the laboratory

10. Biosafety Level 3 laboratory specifications from OSEH

11. Signature pages signed by each researcher indicating successful completion of required JHI and laboratory-specific training for work to be performed in the BSL 3 laboratory.

H. VACCINIA VIRUS VACCINATION

Vaccinia vaccination should not be given for international travel. Vaccinia vaccination is indicated only for laboratory and health care workers directly involved with smallpox or human orthopoxviruses, e.g., monkeypox, vaccinia, cowpox, and others. Principal hazards to laboratory and animal care personnel are ingestion, accident parenteral inoculation, or exposure of mucous membranes or broken skin to droplets or aerosols of infectious fluids or tissues. The ACIP recommends administration of vaccinia vaccine to protect against these hazards.

The following individuals should receive vaccinia within every ten (10) years:

1. Individuals who work directly with non-smallpox human orthopoxviruses or infectious recombinants derived from the human orthopoxviruses including cowpox, vaccinia or vaccinia subspecies; e.g., buffalopox, rabbitpox. No human infections seem to be caused by certain others; e.g., mousepox, camelpox, racoonpox, skunkpox or Uasin gishu virus.

2. All animal care personnel who enter rooms housing animals infected with human orthopoxvirus, or who touch cage litter or cages (prior to decontamination) which have housed infected animals.

3. Certain health care workers involved in human trials of recombinant vaccinia vaccines.

4. Vaccinia vaccination is not required for visitors or service personnel who enter other laboratories where work is conducted only with vaccinia, recombinant vaccinia or cowpox viruses, provided they do not enter the laboratory while work with live viruses is actually in progress.

REGISTRATION OF RESEARCH WITH HBV AND/OR HIV

A. REQUIREMENTS

1. Registration is required for investigators using human blood, tissue, body fluids, and other materials potentially containing HBV or HIV.

2. All research laboratories working with peripheral blood leukocyte cultures, other tissue cultures containing HBV, HIV-I, -II, (HTLV-I, II, III/LAV) suspected HBV, HIV or AIDS-associated retrovirus(es) must register and be inspected by the Office of Safety & Environmental Health.

3. Experimental animal studies involving the use of human HBV or HIV, suspected HIV, AIDS-associated retrovirus(es) or SIV will not be initiated without prior approval from the Biosafety Officer and the Animal Use and Care Committee.

4. Growth and maintenance of HBV or HIV in permissive cell lines must be carried out under work practices described in this document.

5. Large-scale production (one liter quantities or greater) of HBV or HIV and concentrating virus-producing cells must be performed in an approved Biosafety Level 3 facility with HEPA filtration of facility exhaust, when appropriate.

B. INFORMED CONSENT FOR USE OF POSTEXPOSURE ZIDOVUDINE (AZT)

1. The Johns Hopkins Institutions offers the drug zidovudine (AZT) to employees who have a serious exposure to Human Immunodeficiency Virus (HIV, the AIDS virus) while on the job. However, the Institutions do not actively recommend that anyone take AZT for this purpose. The reason is that while AZT works to prolong the lives of patients who are already infected with HIV, it has not been shown that AZT works to prevent HIV infection after an exposure on the job. The scientific reasons for offering AZT, the possible side effects of AZT, and the plan to watch for side effects of AZT and for any signs of HIV infection are outlined below.

2. Several studies show that the chance of becoming infected with HIV following a needlestick injury involving blood from an HIV-infected patient is about 0.4%, or 4 infections for every 1,000 needlestick injuries. The risk from exposure of the mouth, eye, or broken skin is unknown and is probably much lower than the 0.4% figure for needle sticks.

3. AZT works by preventing HIV from reproducing itself inside the cells of the human body. AZT prolongs the lives of patients with AIDS and it slows the course of HIV disease in patients with earlier stages of HIV infection. There are no studies showing that AZT works in preventing HIV infection after an exposure on the job. There are some reports of failure of AZT to prevent HIV infection after such exposures, although the number of reported failures is small. Studies in animals infected with viruses like HIV have shown that AZT may change the course of these infections when AZT is given before or very soon after virus infection. Most animals stayed well longer, but still were infected with the virus.

4. The reason for offering AZT soon after exposures on the job is that it is possible that AZT might prevent HIV infection, although the studies mentioned above do not show that AZT will work when given in this way. The Food and Drug Administration (FDA) has approved AZT for patients already infected with HIV. The FDA has not approved AZT as a treatment to prevent infection after an exposure on the job. Therefore, the use of AZT in this way is considered experimental. We recommend that if the drug is to be given at all, AZT treatment should be started as quickly as possible, within one to four hours of exposure (AZT will not be offered if 72 hours or more has passed from the time of exposure). The AZT dose offered is 200 mg every four hours for 72 hours and then 100 mg every four hours while awake (five times per day) for the following 25 days.

5. There are side effects and other possible risks that should be known. The most common side effects in patients with AIDS who receive AZT are headache, nausea, muscle aches, anemia and low numbers of white blood cells in the blood. Rarely, patients taking AZT develop numbness or tingling in the hands and feet or develop liver damage. Most of these side effects are seen with prolonged use in patients with advanced HIV infection and would not apply to a healthy person taking a short course of the drug.

6. The most common complications in health care workers given AZT for on-the-job exposures are fatigue, trouble sleeping, headaches, nausea and flu-like symptoms. These side effects generally go away quickly when the drug is stopped or if the dose is lowered.

7. The long term effects of AZT in humans are not known. Long term use of the drug in rats and mice produces an increased risk for developing cancer of the vagina. It is not known if AZT can cause harm to unborn children in pregnant women or can influence a woman's ability to have children.

8. Due to these possible side effects and unknown effects on the unborn child, women of childbearing age should have a negative pregnancy test before starting AZT treatment. Also, health care workers who take AZT must agree to not become pregnant or to breast feed while taking the drug and for four weeks after it is stopped (AZT is passed into breast milk). It is possible that taking AZT would not prevent HIV infection, but would instead delay the blood test turning positive. Therefore, those who take AZT should continue to take precautions for one year after exposure.

9. Those who receive AZT treatment must be seen in the Workers' Compensation Clinic as soon as can be scheduled after exposure and at weeks 2, 4 and 6, and at months 3, 6 and 12 after exposure. Blood will be taken every two weeks during AZT treatment and twice more after this has been stopped to check blood counts and liver function tests. If side effects occur, the dose of AZT may be lowered or the

drug may be stopped. Blood tests for HIV will be done at the first visit, and at months 3, 6 and 12.

10. The choice of whether or not to be treated with AZT is up to each individual. The drug will be provided for students of Johns Hopkins University Schools of Medicine and Nursing, for employees of Johns Hopkins Schools of Medicine and Hygiene & Public Health and for employees of The Johns Hopkins Hospital. If you choose to take AZT, you may decide to stop it at any time, however, the Workers' Compensation Clinic should be told of this decision. Your decision to take or to stop AZT will have no effect on your current position or on any other treatment or follow-up that you would receive as a result of this exposure.

11. All your records will be kept confidential within the limits of the law. Documents which identify an individual by name will be kept in a secured file by your physician.

12. If an individual is found to be infected with HIV, they and their sexual partner(s) will be offered information on the test results and how to prevent the spread of the infection. Individuals should also know that it is necessary to report certain communicable diseases, including AIDS, to appropriate state and federal government agencies.

REGISTRATION OF RESEARCH WITH SHEEP OR GOATS

A. BACKGROUND

The Q fever policy was formulated by a special Johns Hopkins committee in 1981 and revised to include the suggestions of The JHU General Counsel, in March 1982. Q fever is a zoonosis caused by the rickettsia, *Coxiella burnetii*. Domestic ungulates such as sheep, cattle, and goats serve as the reservoir of infection for humans and shed the desiccation-resistant organism in urine, feces, milk, and especially in birth products. In man, the illness is generally mild and can be treated with the tetracycline family of antimicrobial agents. However, Q fever hepatitis is often seen and Q fever endocarditis is an uncommon but frequently fatal complication.

Q fever is an occupational hazard among persons working with animals or animal products, and in laboratories working with *C. burnetii*. Recently, Q fever outbreaks have occurred in medical research facilities using sheep or goats as research animals.

B. RECOMMENDED CONTROL MEASURES FOR Q FEVER

1. All investigators using sheep or goats in their experiments shall register with The Johns Hopkins Institutions Biosafety Officer. The user shall describe;

    a. the plan to protect employees during use of sheep and goats, including the handling of products of conception and excrement,

    b. the room(s) where sheep or goats will be housed,

    c. the room(s) where sheep or goat related biological samples will be used, and

    d. a list of all professional personnel, employees and students involved in the project who will come into contact with the sheep or goat products.

2. The Biosafety Officer will send a "To Whom It May Concern" letter to the principal investigator for forwarding to the granting agency when appropriate.

3. All professional personnel, employees and students coming into direct contact with sheep and goats or sheep and goat products of conception will be included in the medical surveillance program at Occupational Health Services.

4. Employee serum samples shall be collected by Occupational Health Services for future use as a baseline reference. Serum samples shall be collected at the new employment physical.

5. Occupational Health Services will take an additional serum sample upon termination of employment or transfer to a position which does not involve direct contact with sheep or goats or their products. It is the responsibility of the principal investigator to notify Occupational Health Services that an employee is leaving and to make arrangements for the post-employment medical examination.

6. All employees and students entering sheep and goat holding areas, laboratories and surgical facilities shall be informed of the Q fever hazard and shall receive written instructions on safe work practices to minimize the Q fever hazard.

7. All personnel involved with sheep and goats or products of conception from sheep and goats shall be informed of the symptoms of Q fever and shall be instructed to contact the Workers' Compensation Clinic if they experience the symptoms.

8. In the event of a failure or breakdown in any of the systems or procedures described herein, investigators shall report incidents to the Workers' Compensation Clinic as soon as possible. The report must be in writing on the JHU Report of Incident Form.

REGISTRATION OF RESEARCH WITH RECOMBINANT DNA

Research involving recombinant DNA requires strict adherence to the most current NIH Guidelines for Recombinant DNA Research as published in the Federal Register, and any local, state or federal regulation promulgated for Recombinant DNA activities.

A. REGISTRATION FORM

All principal investigators conducting recombinant DNA research are required to file the JHI Recombinant DNA Registration Form.

B. REVIEW PROCEDURE

This information is required for evaluation of each proposal for compliance with NIH Recombinant DNA guidelines.

The Biosafety Officer will review all proposals to identify those covered by the NIH Guidelines and those which are exempt. The Recombinant DNA Committee, a subcommittee of the Institutional Biosafety Committee, will review all non-exempt proposals. An annual survey of recombinant DNA activity, by month of last annual registration, will be used to update these files. The investigator will be informed of the containment conditions established by the Recombinant DNA Committee and/or the Biosafety Officer.

The review the of recombinant DNA proposals will provide:

1. Documentation that the proposed project is exempt from the NIH Guidelines, or

2. Documentation that the proposed project covered by the NIH Guidelines is in compliance with the Guidelines, and

3. Assurance that the facilities will be in compliance with the NIH Guidelines.

4. A "To Whom It May Concern" letter of will be sent to the principal investigator for forwarding to the granting agency when appropriate.

5. No project involving recombinant DNA research may be implemented without registration with the Biosafety Officer.

6. No major changes in recombinant DNA experiments may be implemented without notification of the Biosafety Officer. Minor changes need not be reported until the annual update of recombinant DNA activity.

7. A new or revised application is required for major changes in the recombinant DNA aspects of an ongoing project, such as a change in the;

   a. hosts or vectors.

   b. donor species or nature of the DNA segment.

   c. location of experiments, or

   d. responsible investigator.

C. REVIEW OF RECOMBINANT DNA RESEARCH WITH MODERATE RISK BIOLOGICAL AGENTS OR MATERIALS AT BIOSAFETY LEVEL 1 or 2

1. Complete the Recombinant DNA Form and the Potentially Pathogenic / Oncogenic Agent or Material Registration Form and forward them to the Biosafety Officer.

2. Principal investigators are to submit a research protocol, including a construct of the recombinant molecule(s), to the Biosafety Officer.

3. Arrange with the Biosafety Officer for an on-site inspection of practices and containment facilities.

4. The Biosafety Officer will:

   a. Register the research protocol and send a letter containing a unique registration number, recombinant DNA Guideline reference, and assigned Biosafety Level for the research.

   b. Submit the information for review by the Recombinant DNA Committee.

D. REVIEW OF RECOMBINANT DNA RESEARCH INVOLVING BIOSAFETY LEVEL 3 FOR HIGH RISK BIOLOGICAL AGENTS OR MATERIALS OR RECOMBINANT DNA RESEARCH INVOLVING HUMAN SUBJECTS

1. Complete the Recombinant DNA Form and the Potentially Pathogenic / Oncogenic Agent or Material Registration Form and forward them to the Biosafety Officer.

2. Principal investigators must submit a research protocol, including a construct of the recombinant molecule(s), to the Biosafety Officer.

3. Principal investigators must submit a Standard Operating Procedure Manual to the Biosafety Officer for all research involving biosafety level 3 containment.

4. Principal investigators must submit a Standard Operating Procedure Manual for research involving human gene therapy to the Biosafety Officer.

5. Arrange with the Biosafety Officer for an on-site inspection of practices and containment facilities.

6. The Biosafety Officer will:

   a. Register the research protocol and send a letter containing a unique registration number, recombinant DNA Guideline reference, and assigned Biosafety Level for the research.

   b. Submit a report for review by the Recombinant DNA Committee and/or the Institutional Biosafety Committee.

7. The protocol and inspection report will be reviewed and approved by the Joint Committee on Health, Safety and Environment.

8. Results of review will be forwarded to; the investigator, the department director and the dean of the appropriate university division.

REGISTRATION OF RESEARCH WITH NON-HUMAN PRIMATES

INTRODUCTION

Research with non-human primates is registered with the Office of Safety and Environmental Health to ensure that individuals working with the animals receive appropriate medical surveillance, particularly the PPD skin test. If primates acquire tuberculosis, the disease may be transmitted to humans, or visa versa.

A. TUBERCULOSIS

Tuberculosis is a chronic disease caused by bacteria of the genus *Mycobacterium*. The most common form of the disease affects the lungs. Infection of other organs is much less common. The main source of infection for humans in the United States is other infected humans. In under-developed countries cattle may also be commonly infected and serve as a source of infection for humans. The practice of milk pasteurization coupled with test and slaughter programs for cattle have eliminated this source of infection in this country.

Non-human primates (monkeys) are very susceptible to tuberculosis, and most commonly die if infected. The most common source of infection for monkeys is from infected humans or other monkeys. Since many of the monkeys used in this institution are caught in the wild, there is the possibility that they may catch tuberculosis from the humans with which they come into contact.

Tuberculosis in Non-Human Primates: While tuberculosis is a potential problem for humans in the hospital or research setting, this disease can also be highly infectious in a non-human primate colony. All monkeys coming into the institution are quarantined for a minimum of 42 days to allow for three tuberculin tests at two week intervals. After the quarantine period, all monkeys are periodically tuberculin tested, usually at 3 month intervals.

1. Tuberculin Testing
   In order to protect the humans who work or come into contact with non-human primates; and to protect the monkeys who come into contact with humans, each is tuberculin tested on a routine basis.

   At six month intervals all individuals who work with non-human primates will be notified of the time and place for tuberculin testing.

2. Personal Protective Equipment
   What can be done to minimize the possibility of catching tuberculosis from a monkey? The simplest preventive measure is to wear a face mask and gloves when handling, or working with monkeys.

B. HERPESVIRUS-B IN MACAQUE MONKEYS

1. Background

   All macaque monkeys are potential carriers of herpesvirus-B, also called herpes simiae. In this institution, the two macaque species commonly used are rhesus monkeys (Macaca-mulatta) and cynomolgus monkeys (*Macaca fasicularis*). This virus is not found in non-macaca species of monkeys such as the african green, squirrel, or baboon

2. Human Infection With Herpesvirus-B

   Herpesvirus simplex is a human virus that commonly causes oral cold sores in people. Herpesvirus-B acts similarly in the monkey, it sometimes causes a cold sore-like lesion but generally causes no obvious physical problem for the monkey. The problem comes when a human becomes infected with the macaque virus. While human infection is very rare, the unfortunate consequence of such an infection is usually death or severe and lasting neurologic disease.

   Humans become infected with Herpesvirus-B usually by receiving a bite or a scratch from an infected monkey who is shedding virus, from contact with tissues from an infected monkey, or by injury from a cage or similar item that has been contaminated with material from an infected monkey.

3. Antibody testing

   Monkeys are often tested for the presence of antibody to Herpesvirus-B. However, animals with negative titers are still capable of being infected and shedding virus.

   Therefore, the safe thing to do is to treat all macaques as though they are infected with this virus.

4. Personal Protective Equipment

   The best way to prevent infection with this agent is to use common sense when working with monkeys and to utilize protective devices.

   a. Always wear face masks, long-sleeved laboratory coats or other clothing, and rubber gloves when working with monkeys.

   b. Unless absolutely necessary, never handle an awake macaque. Use a squeeze cage and a restraint drug such as ketamine to catch an animal.

   c. If you have to work with an awake animal, always use care when working near the head or close enough for the monkey to grab you.

5. Response to a Bite or Scratch

   a. If you do receive a bite or scratch wound from a monkey; or from a piece of equipment with which the animal or animal tissues has had contact, *STOP WORK*, return the monkey to its cage, institute first aid immediately. Each primate housing area, and laboratory has been equipped with a "Monkey Injury Kit". Follow the instructions that are inside the kit. You should familiarize yourself with the instructions in advance.

   b. Notify Animal Services of the identification number of the monkey and its location so that a veterinarian can examine the animal and take appropriate steps to ascertain the potential hazard. Following initial first aid, go to the Workers' Compensation Clinic.

6. First-Aid for Macaque Associated Wound
   Each facility housing macaque monkeys, and each laboratory using such animals is equipped with a "Monkey Injury Kit". Following a monkey associated wound immediately open the kit.

   Inside you will find the following items:
   - two screw-top culture tubes numbered "1" and "2"
   - two packages of sterile, cotton topped applicators
   - one, 1 ounce brown bottle of bleach
   - one, sterile povidone-iodine impregnated scrub brush
   - 4x4 inch gauze sponges
   - one roll of adhesive tape

   a. IF YOUR EYES, NOSE OR MOUTH HAVE BEEN EXPOSED, IRRIGATE THE SITE WITH STERILE SALINE OR RAPIDLY FLOWING WATER FOR 15 MINUTES and go to the Workers' Compensation Clinic.

   b. ONLY FOLLOW THESE INSTRUCTIONS FOR WOUNDS OF THE SKIN. DO NOT USE FOR EYE, NOSE OR MOUTH CONTAMINATION

      1) Open the Injury Kit and remove the contents.

      2) If possible, take one sterile cotton swab, insert into wound and rotate gently. Remove top from screw-top tube "1", and insert swab part way inside. Break off wooden applicator so that the handled portion stays outside of the tube. Replace top on tube.

3) Dump the contents of the brown bottle into the Kit container, and fill with water to the black line. Then using the 4x4 gauze sponges, soak the wound with the prepared solution for at least five minutes.

4) Open the scrub brush package and gently but thoroughly scrub the wound with the sponge brush and running water for 15 minutes.

5) After washing the wound, cover with gauze, secure with adhesive tape, and go to the Workers' Compensation Clinic. Take the tube labeled "2" and the second package of sterile applicators with you.

STANDARD PRACTICES, TRAINING AND PRECAUTIONS

The objective of physical containment of biohazardous materials is to confine biological agents or material and/or organisms containing recombinant DNA molecules, and thus reduce the potential of injury to laboratory workers and persons outside of the laboratory. Physical containment is achieved through the use of appropriate laboratory practices, containment equipment and special laboratory design. Emphasis must be placed on primary containment which is provided by laboratory practices and containment equipment. Special laboratory design provides secondary containment against accidental release of organisms or materials outside the laboratory or to the environment.

The combination of laboratory practices, containment equipment and special laboratory design used to achieve physical containment includes approaches to containment of pathogenic or oncogenic organisms and organisms containing recombinant DNA. The standards are based on the four levels of containment identified by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH) in their publication, *Biosafety in Microbiological and Biomedical Laboratories*, which encompass the elements of physical containment described in the NIH Guidelines for Recombinant DNA Research with some editorial changes.

A. All personnel directly or indirectly involved in experiments with biological agents and materials or recombinant DNA must receive adequate instruction including, but not limited to;

    1. The biology of the organism(s) or material(s) used in experiments with emphasis on potential biohazards,

    2. Microbiological aseptic techniques, and

    3. Proper techniques for decontamination / disinfection.

B. There should be strict adherence to good microbiological practice in all experiments.

C. All research groups must have a written emergency plan which describes the procedures to be followed if an accident contaminates personnel, the laboratory, or the environment.

D. Vaccination may be appropriate for research with selected disease agents.

E. Serological monitoring and periodic medical examinations, where appropriate, will be provided by Occupational Health Services.

F. All accidents and illnesses must be reported promptly to the Workers' Compensation Clinic.

G. The selection of an appropriate biosafety level for work with a particular agent and/or animal is dependent upon a number of factors. The most important of these include:

1. The virulence, pathogenicity, biological stability, and communicability of the agent;

2. The nature or function of the laboratory or activity;

3. The quantity and concentration of the agent;

4. The endemicity of the agent; and,

5. The availability of effective vaccines or therapeutic measures.

If a combination of increasingly stringent primary and secondary containment procedures and facilities are used, laboratory studies and manipulations can be safely conducted on agents that are correspondingly more hazardous.

H. In general, the biosafety level used for activities with infectious agents or infected animals should be commensurate with that required for the agent of highest virulence known or likely to be encountered in the course of contemplated work.

UNIVERSAL PRECAUTIONS GUIDELINES FOR RESEARCH LABORATORIES

A. DEFINITIONS

The Centers for Disease Control (CDC) / National Institutes of Health (NIH) Universal Precautions Guidelines cover all blood, tissue, body fluids from inside the body, and any material which contains blood that can be seen. These materials must be considered infectious for Hepatitis B and AIDS and must be handled under conditions which reasonably preclude cutaneous, oral, or parenteral exposure to personnel.

Universal Precautions, sometimes termed Blood and Body Fluid Precautions, must be used for handling **ALL** specimens covered by the guidelines from **ALL** patients.

Universal Precautions Guidelines state that all blood, tissue, body fluids from inside the body, and any material which contains blood you can see are considered potentially infectious for hepatitis and AIDS and must be handled under conditions which reasonably preclude cutaneous, oral, and parenteral exposure to personnel. Work with these materials is often registered at biosafety level 2.

Sputa should be considered potentially infectious for tuberculosis and should be handled under conditions which reasonably preclude generation of aerosols and also contain previously generated aerosols. Work with sputum is often registered at biosafety level 3.

B. PRECAUTIONS

1. If in the course of diagnostic or other laboratory examinations there is evidence that the materials being studied contain an agent of higher or lower risk than expected, the biosafety level should be raised or lowered accordingly, with the advice of the Biosafety Officer.

2. The following recommended precautions are advised for persons performing laboratory tests or studies on clinical specimens or other potentially infectious materials such as tissue culture, embryonated eggs, or tissue from animals inoculated with human blood, tissue, or body fluids:

   Biosafety Level 2 (BSL 2) standards and special practices, containment equipment, and facilities are recommended for activities involving all clinical specimens, body fluids, and tissues from humans or from infected or inoculated laboratory animals.

3. Use of syringes, needles and other sharp instruments shall be avoided if possible. Used needles and disposable cutting instruments must be discarded into an approved puncture-resistant container. Needles shall not be re-sheathed, bent, broken, removed from disposable syringes, or otherwise manipulated by hand.

4. Protective gloves shall be worn by all personnel engaged in activities that may involve direct contact of their skin with potentially infectious specimens, cultures, or tissues.

5. Gloves shall be carefully removed and changed when they are visibly contaminated.

6. Personnel who have dermatitis or other lesions on their hands and who may have indirect contact with potentially infectious material shall also wear protective gloves.

7. Hand washing with mild soap and water immediately after infectious materials are handled, after work is completed, after contaminated gloves are removed, and after removal of gloves for any reason shall be a routine practice.

8. Generation of aerosols, droplets, splashes, and spills shall be avoided. A biological safety cabinet shall be used for all procedures that might generate aerosols or droplets and for all infected cell-culture manipulations.

9. Fluorescence-activated cell sorters generate droplets that could potentially result in infectious aerosols. Translucent plastic shielding between the droplet-collecting area and the equipment operator should be used to reduce this risk. HEPA-filtered air exhaust systems may also be required when sorting live cells. Please contact the Biosafety Officer for further information.

10. The use of plastic labware is encouraged to reduce breakage and minimize the risk of puncture wounds.

11. Activities such as producing research laboratory scale amounts of HIV, manipulating concentrated virus preparations, and conducting procedures that may produce aerosols or droplets shall be performed in a BSL 2 facility with the additional practices and containment equipment recommended for BSL 3.

12. Activities involving industrial-scale, large-volume production (volumes of one liter or greater) or high concentrations or manipulations of concentrated HIV shall be conducted in a BSL 3 facility using BSL 3 practices and equipment.

13. BSL 2 practices, containment equipment, and facilities for animals are recommended for activities involving non-human primates and any animals experimentally infected or inoculated with HIV. Because laboratory animals may bite, throw feces or urine, or expectorate, all animal services personnel, investigators, technical staff, students, and other persons who enter these animal rooms shall wear coats, protective gloves, coveralls or uniforms, and when appropriate, face shields or surgical masks and eye protection to protect their skin and mucous membranes of their eyes, nose, and mouth. Only non-human primates

directly involved in the experiments being conducted are permitted in the laboratory.

14. All laboratory glassware, disposable material, and waste material known to have come into contact with HIV culture, or any other microbial culture, should be decontaminated, preferably in an autoclave, before it is washed, discarded, etc. A method of disposing of other solid wastes is the biohazard box.

15. Laboratory workers shall wear laboratory coats, gowns, or uniforms when working with HIV or with material known or suspected to contain HIV. Clothing that becomes contaminated with HIV preparations should be decontaminated before being laundered or discarded. Laboratory personnel must remove laboratory clothing before leaving the laboratory.

16. Work surfaces shall be decontaminated with an appropriate chemical germicide when procedures are completed, when surfaces are overtly contaminated, and at the end of each work day. Many commercially available chemical disinfectants can be used for decontaminating laboratory work surfaces, laboratory instrument surfaces, spot cleaning of contaminated laboratory clothing, and for spills of infectious materials. Prompt decontamination of spills should be standard practice.

17. If the outside of the specimen container is visibly contaminated, it should be cleaned with a disinfectant such as a 1:10 dilution of liquid household bleach with water. (Household bleach contains 5.25% or 52,500 ppm sodium hypochlorite). All blood specimens should be placed in a secondary container, such as a portable cooler, for transport. The container and bag should be examined carefully for breaks or cracks and should be routinely decontaminated.

18. Blood spills should be cleaned up promptly with a disinfectant solution, such as sodium hypochlorite (see above) and detergent. Articles soiled with blood should be discarded into a red bag-lined biohazard box.

19. Universal precautions are recommended for handling all human blood specimens for hematologic, microbiologic, chemical, and serologic testing; these are the same precautions for preventing transmission of all bloodborne infections including hepatitis B. It is not certain how effective 56-60º C heat is in destroying HIV in serum, but heating small volumes of serum for 30 minutes at 56º C before serologic testing may, under some circumstances, reduce residual infectivity. Such treatment causes some false-positive results in HIV enzyme immunoassays and may also affect some biochemical assays performed on serum.

20. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL 2.

21. Medical surveillance programs should be in place in all laboratories that test specimens, do research, or produce reagents involving HIV. The nature and scope of a surveillance program needed for a particular laboratory or project should be discussed with Occupational Health Services.

22. Other primary and opportunistic pathogenic agents may be present in the body fluids and tissues of persons infected with HIV. Laboratory workers shall follow accepted biosafety practices to ensure maximum protection against inadvertent laboratory exposure to agents that may also be present in clinical specimens.

23. The laboratory director or designated laboratory supervisor is responsible for carrying out the biosafety program in the laboratory. The laboratory director or designated supervisor, in consultation with the Biosafety Officer, shall establish the biosafety level for each component of the work to be done and shall ensure that facilities and equipment are adequate and in good working order. The laboratory director or designated laboratory supervisor shall ensure that appropriate initial and periodic training is provided to the laboratory staff, and that recommended practices and procedures are strictly followed.

24. Access to the laboratory must be limited by the principal investigator. The door to the laboratory must be closed and posted with the universal biohazard symbol. Only persons who have been advised of the potential hazards of the work and meet specific entry requirements may enter the laboratory. Persons who are at increased risk of acquiring infection are not permitted in the laboratory.

HBV and/or HIV AGENT SUMMARY STATEMENTS

These publications, available at the Office of Safety & Environmental Health, are good reference sources:

A. *1988 Agent Summary Statement for Human Immunodeficiency Virus and Report on Laboratory Acquired Infection With Human Immunodeficiency Virus*. MMWR, April 1, 1988/Vol.37/No. S-4 Supplement.

B. *Guidelines for Prevention of Transmission of Human Immunodeficiency Virus and Hepatitis B Virus to Health-Care and Public-Safety Workers*. MMWR, June 23, 1989/Vol. 38/No. S-6.

C. *H.I.V. Infection Control Guidelines*, Maryland Governor's Advisory Council on Aids 5/89.

D. *Occupational Exposure to Bloodborne Pathogens; Final Rule*. Occupational Safety and Health Administration. 29 CFR Part 1910.1030. Federal Register, vol. 56, No. 235, December 6, 1991, p. 64175-64182.

E. *Enforcement Procedures for the Occupational Exposure to Bloodborne Pathogens Standard, 29 CFR 1910.1030*. OSHA Instruction CPL 2-2.44C, March 6, 1992.

F. *Guidelines for Protecting the Safety and Health of Health Care Workers*, U.S. Department of Health & Human Services, National Institute for Occupational Safety & Health, DHHS (NIOSH) Publication No. 88-119, September, 1988.

G. *Public Health Service Statement on Management of Occupational Exposure to Human Immunodeficiency Virus, Including Considerations Regarding Zidovudine Postexposure Use*, Morbidity & Mortality Weekly Report, vol. 39, no. RR-1, January 26, 1990.

H. *Working Safely With HIV in the Research Laboratory; Biosafety Level 2/3*, Occupational Safety and Health Branch, NIH Division of Safety, June, 1988.

I. *Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Settings, with Special Focus on HIV-Related Issues*, Morbidity & Mortality Weekly Report, vol. 39, no. RR-17, December 7, 1990.

J. *Johns Hopkins Institutions Bloodborne Pathogens Professional Study Guide*, February, 1994.

Q FEVER PRECAUTIONS

A. BACKGROUND

The Q fever policy was formulated by a special committee in 1981 and revised to include the suggestions of The JHU General Council, in March 1982. Q fever is a zoonosis caused by the rickettsia, *Coxiella burnetti*. Domestic ungulates such as sheep, cattle, and goats serve as the reservoir of infection for humans and shed the desiccation-resistant organism in urine, feces, milk, and especially in birth products. In man, the illness is generally mild and may be treated with the tetracycline family of antimicrobial agents. However, Q fever hepatitis is often seen and Q fever endocarditis is an uncommon but frequently fatal complication. Q fever is an occupational hazard among persons working with animals or animal products, and in laboratories working with *C. burnetti*. Recently, Q fever outbreaks have occurred in medical research facilities using sheep as research animals.

B. RECOMMENDATIONS

1. The site of choice for conduct of sheep research should be an isolated setting apart from hospital research and patient care areas.

2. It is possible to conduct research with sheep which are known or suspected to be infected provided such research is performed in a containment facility or in an isolated setting where only at-risk workers are admitted. When appropriate, selected workers could be skin tested.

C. RATIONALE

These recommendations suggest measures for reducing the risk of human infection with Q fever in facilities where sheep are used for research. They can neither encompass every possible circumstance in which personnel might be exposed to Q fever-infected sheep, nor can their implementation guarantee that transmission will not occur. Depending on the specific research facility, more intensive or less intensive control measures may be appropriate. Although some of the recommendations are not supported by controlled epidemiologic trials, they do reflect the current understanding of the transmission and control of Q fever.

D. REGISTRATION

All investigators using sheep or goats in their experiments shall register with the Biosafety Officer, Office of Safety and Environmental Health, using the form entitled "Registration of research with sheep and goats". The user shall describe; a) the plan for the use of sheep or goats, including the handling of products of conception and excrement, b) the room(s) where sheep or goats will be housed, c) the room(s) where sheep or goat related biological

samples will be handled, and d) a list of all professional personnel, employees and students involved in the project who will come into contact with the sheep or goat products.

E. MEDICAL SURVEILLANCE

1. All professional personnel, employees and students coming into direct contact with sheep or goats or with sheep or goat products of conception will be included in the medical surveillance program. Employee serum samples shall be collected by Occupational Health Services as a baseline reference. Serum samples shall be collected at the employment physical to serve as a baseline reference if needed. A post-employment serum sample shall be taken by Occupational Health Services at termination of employment or transfer to a position which does not involve direct contact with sheep or goats or their products. It is the responsibility of the principal investigator to notify Occupational Health Services that an employee is leaving and to make arrangements for the post-employment medical examination and serum sampling.

2. All employees and students entering sheep and goat holding areas, laboratories and surgical facilities shall be informed of the Q fever hazard and shall receive written instructions on safe work practices to minimize the Q fever hazard.

3. All personnel involved with sheep and goats or products of conception from sheep and goats shall be informed about the symptoms of Q fever and shall be instructed to contact the Occupational Health Services if they experience the symptoms. Accidents and incidents shall be reported to the Workers' Compensation Clinic.

4. In the event of a failure or breakdown in any of the systems or procedures described herein, investigators shall report incidents to the Biosafety Officer, Office of Safety and Environmental Health as soon as possible. Any such report must be in writing and shall include; a) the nature of the incident, b) the date and location of the incident, c) the names of all professional personnel, employees and students involved in the incident, and d) what measures, if any, were taken to prevent further incidents.

F. TRANSPORT OF ANIMALS

Transport of all sheep and goats, pregnant or not, outside of posted Q fever hazard areas shall be in enclosed, ventilated carts which will contain any Q fever rickettsia agents within the cart enclosure. Air intakes and exhaust ports shall be fitted with an appropriate filter. These carts shall be labeled and reserved for the exclusive transport of sheep and goats. Carts shall not be opened or left unattended outside of posted Q fever hazard areas. Transport carts shall be decontaminated after use. Transport of sheep and goats within buildings shall be by established routes. Sheep and goats shall not be transported, under any conditions, on elevators designated for patient or visitor use.

G. PERSONNEL PROTECTION

1. Personal protective equipment shall be provided and worn by all personnel working in animal rooms, laboratories, and surgical facilities.

2. The clothing for animal caretakers shall include full body protective clothing, head cover, gloves, boots, and an approved respirator or mask. Protective clothing must remain in the containment facility. Disposable protective clothing must be decontaminated before disposal or properly red-bagged and incinerated.

3. Laboratory or surgical personnel must wear wrap-around or solid-front gowns or uniforms when entering posted areas (Front-button laboratory coats are not suitable). The use of respirators or masks and gloves is recommended in posted areas. Protective clothing must remain in the facility and must be decontaminated before being laundered. Disposable protective equipment shall be autoclaved and then discarded into a red bag-lined biohazard box.

4. The use of disposable personal protective equipment, i.e. surgical gowns, Tyvek^{Tm} suits, face masks, etc., is recommended. It is recommended that animal handlers and technical operations personnel be provided a separate clean clothing change for use with sheep or goats. Clothing may be reused if personnel are not involved with sheep or goat products of conception on a full time basis. Obviously soiled or overtly contaminated clothing should be removed, wetted down with a disinfectant and placed in a container for autoclaving and incineration.

5. All personal protective equipment shall be distinguished by a unique color or visible marking to be immediately recognizable as equipment that should not be used outside of a containment facility.

6. An appropriate closed container should be provided to hold discarded protective clothing prior to autoclaving and incineration.

7. Personnel should use soap to wash their hands for ten to fifteen seconds, particularly after removing gloves and before leaving containment areas. Washing with mild soap is sufficient if good handwashing technique is used.

H. WASTE DISPOSAL

1. All sheep or goat related debris from animal pens and laboratories - litter, sheep carcasses, excrement and products of conception shall be disposed of safely. Some materials shall be discarded into red bag-lined biohazard boxes for incineration. Other materials may be autoclaved in appropriate containers and discarded into a red bag-lined biohazard box for incineration.

2. The clean-up of animal debris shall be done in a manner which will produce the least amount of airborne materials (aerosols).

I. DECONTAMINATION

1. All areas and equipment involving any contact with sheep or goats or products of conception from sheep or goats shall be cleaned and disinfected on a regular basis and immediately after each operation.

2. Special cleaning and disinfecting is required for machines used in Q fever hazard areas which are shared with other areas. The manufacturer should be consulted if high tech machines in involved, but in general, a good cleaning with a hospital grade disinfectant-detergent registered with the EPA should be carried out on all accessible surfaces, including legs and wheels.

3. Disinfection may be performed in surgical and laboratory areas with diluted chlorine bleach solution (a 1 to 10 dilution of liquid household bleach with tap water), a fresh 5% solution of hydrogen peroxide, or a 1:100 dilution of Lysol^{Tm}.

4. Some disinfectants, such as ethyl alcohol, 1% phenol, 1% formalin and quaternary ammonium compounds, are not effective.

5. Dr. Frank Gohr, University of California, in 1979, found the most effective, practical and economical germicide for cleaning potential Coxiella burnetti contaminated areas and equipment relatively free of organic deposits was standard bleach diluted 1:100 with water (1 ml bleach, 99 ml water) to which was added 1 gram of a neutral detergent such as All^{Tm} laundry detergent.

6. If heavy organic material is present (sheep manure, shavings, heavy blood or tissue contamination), use chlorine bleach diluted 1:10 with water (1 ml bleach, 9 ml water) with 1% vol/vol added detergent. Contact time should be at least 20 minutes. The germicidal solution should be mixed daily and checked to assure a pH of 7. Good scrubbing may be needed to assure proper germicide contact.

BIOSAFETY LEVEL 1 GUIDELINES

Biosafety Level 1 (BSL 1) is suitable for work involving agents of no known or of minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science. Refer to Section III -- Recombinant DNA Guidelines.

BIOSAFETY LEVEL 2 GUIDELINES

Biosafety Level 2 (BSL 2) is similar to Biosafety Level 1 and is suitable for work in clinical, diagnostic, teaching, research or production facilities involving agents of moderate potential hazard to personnel and the environment.

It differs in that; (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, (2) access to the laboratory is limited when work is being conducted, and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment. Refer to Section III -- Recombinant DNA Guidelines.

BIOSAFETY LEVEL 3 GUIDELINES

Biosafety Level 3 (BSL 3) is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the aerosol route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. No children are permitted in the laboratory.

The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional air flow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy Biosafety Level 2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices", and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made by the laboratory director on advice of the Biosafety Officer. A specific facility operations manual is prepared, reviewed by the Biosafety Officer, and adopted. Refer to Section III -- Recombinant DNA Guidelines.

ANIMAL BIOSAFETY LEVELS

Laboratory animal facilities, operational practices, and quality of animal care must meet applicable standards and regulations. Also, appropriate species must be selected for animal experiments (Refer to *Guide for the Care and Use of Laboratory Animals*, HHS Publication No. 86-23, Rev. 1985, and *Laboratory Animal Welfare Regulations*, 9 CFR, Subchapter A, Parts 1, 2, and 3). Refer to Section III -- Recombinant DNA Guidelines.

BIOSAFETY REFERENCES

1. *Classification of Etiologic Agents on the Basis of Hazard*. (4th Edition, July, 1974). U.S. Department of Health, Education, and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

2. *Biosafety in Microbiological and Biomedical Laboratories*, Centers for Disease Control, Atlanta, Georgia, National Institutes of Health, Bethesda, Maryland, U.S. Department of Health and Human Services, Public Health Service, HHS Publication No. (CDC) 93-8395, 3rd Edit., May 1993.

3. USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

4. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses*. (October, 1974), U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790.

5. *Guidelines for Protecting the Safety and Health of Health Care Workers*. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, and National Institute for Occupational Safety and Health {Division of Standards Development & Technology Transfer, 4676 Columbia Parkway, Cincinnati, OH, Telephone (513) 533-8287}; DHHS (NIOSH) Publication No. 88-119, September, 1988.

6. *Radiation Safety Manual*, The Johns Hopkins Medical Institutions, Revised July, 1990.

7. *Safety & Emergency Procedure Manual*, The Johns Hopkins Hospital, Revised August, 1992.

8. *Use of Experimental Animals at the Johns Hopkins University*, The Johns Hopkins University School of Medicine, Division of Comparative Medicine, Revised September, 1992.

9. *Laboratory Biosafety Guidelines*, Medical Research Council of Canada and Laboratory Centre for Disease Control, Health Protection Branch, Health and Welfare Canada, Cat. No. MR 21-1/1990E, 1990.

10. *Johns Hopkins University Safety Policy and Procedure Manual*, Johns Hopkins University, Revised August 1994.

11. *Biohazards Management Handbook*, Second Edit., D.F. Liberman, Marcell Dekker, Inc., New York, 1995.

12. *Laboratory Safety Principles and Practices*, Second Edit., D. Fleming, J. Richardson, J. Tulis, D. Vesley, ASM Press, Washington, DC, 1995.

BIOSAFETY LEVEL 3 FACILITY SPECIFICATIONS

A. PERFORMANCE

Laboratory must be effectively air tight and liquid tight.

B. LOCATION

1. Lab separated from unrestricted traffic.
2. Access to lab through two sets of doors.
3. Access to laboratory from an anteroom or change room.

C. FLOORS

1. Integral cove preferred.
2. Seamless or welded vinyl coving sealed to the floor with silicone.

D. WALLS

1. Washable and resistant to detergents and disinfectants, ie., formaldehyde.
2. Durable paint such as epoxy or formica or tile surface.

E. CEILING

1. Monolithic construction (ie., gypsum board, not removable tiles).
2. Same finish as walls.
3. Any access to HVAC, plumbing, or electrical above ceiling must be through access doors that are closed and sealed.

F. ACCESS ZONE

Passage through two sets of doors (anteroom, change room) is mandatory.

G. DOORS

1. Self-closing.
2. Inward opening.

3. No gaskets around door jams.

H. WINDOWS

Closed and sealed.

I. PLUMBING

1. All penetrations are horizontal.

2. Penetrations are caulked with fire retardant seal.

3. No drainpipes or other plumbing penetrates the floor.

J. VACUUM SYSTEM

1. Independent of building central vacuum system.

2. HEPA filtered exhaust.

K. ELECTRICAL

1. All outlets surface-mounted.

2. No junction boxes set into wall.

3. Surface conduit preferred.

4. Wall or ceiling penetrations of electrical cable are to be kept to a minimum and sealed with fire retardant material (ie., Fireseal).

5. Each biological safety cabinet shall have independent circuits.

L. LIGHT FIXTURES

Electrical connections to fixture made with either sealed penetrations or surface mounted conduit.

M. LAB FURNITURE

1. Sturdy.

2. Spaces between benches and equipment for cleaning access.

3. Bench tops impervious to water.

4 Bench tops resistant to acids, alkalis, organic solvents.

5 Bench tops resistant to moderate heat.

N. <u>HAND WASHING SINK</u>

1. In laboratory, near exit door.

2. Wrist, elbow, foot, automatically operated.

O. <u>EYE, FACE AND BODY SPRAY FIXTURE</u>

1. Located in laboratory near exit door.

2. Near or on hand washing sink.

P. <u>AUTOCLAVE</u>

1. Must be available on same floor as the laboratory.

2. Preferred within lab or with pass-through to anteroom or glassware processing area with bioseal at wall.

3. Autoclave drain must be inside the laboratory.

4. Penetration of drain through the floor, if essential, must be sealed.

Q. <u>VENTILATION</u>

1. Ducted exhaust must be discharged directly outside the building with dedicated primary and secondary backup fans.

2. Exhaust is not to be recirculated to any other part of building.

3. Exhaust discharge outside must be away from occupied areas and building air intakes.

4. Exhaust discharge should be HEPA filtered with a bag-in-bag-out prefilter and bag-in-bag-out HEPA filter housings.

5. Ventilation system (supply & exhaust) must contain 100% shut off dampers to isolate the laboratory from other areas of the building.

6. Supply and exhaust systems must be interlocked so that supply shuts off when exhaust is off.

R. BIOLOGICAL SAFETY CABINETS

1. Class II, Type B3 recommended.

2. HEPA-filtered exhaust through thimble connection.

3. Thimble exhaust shall not interfere with the air balance of the room.

4. Ceiling around thimble should allow opening of the thimble door(s) for certification tests.

5. Exhaust air flow balance in thimble connection shall be 150% of the biological safety cabinet manufacturer's exhaust specification.

6. All utility lines to the BSC shall be installed behind the cabinet.

7. The BSC shall be set six inches out from the real wall.

8. Gas line to the BSC shall have access to a shutoff valve with handle on either the left or right-hand side of the cabinet, depending on which side the internal gas valve is located.

9. Gas supply line shall have a pipe union between the shutoff valve and the connection to the BSC.

S. CHEMICAL FUME HOOD

1. The sash opening shall be set to 18 inches and the exhaust air shall be balanced to provide an airflow of 100 linear feet per minute at the work opening.

2. Flammable storage cabinet and/or acid-base cabinet shall be vented into the chemical fume hood with pipe extending from a fire arrestor screen at the rear of the flammable storage cabinet and extending behind and four inches above the bottom of the rear baffle at the rear of the fume hood.

T. AIR PRESSURE DIFFERENTIAL

1. Anteroom shall be at least 50 CFM negative with respect to corridor.

2. Laboratory shall be at least 50 CFM negative with respect to anteroom.

U. DIRECTIONAL AIRFLOW

Ducts to be installed so air flows from area of least hazard to area of greatest hazard potential.

V. AIR SUPPLY VENTS

1. Vents shall be located away from the face of biological safety cabinets and CO_2 incubators.

2. The vents shall be four way, three way, two way, or one way depending on the locations of areas of greatest hazard potential.

3. Low velocity, large surface area supply vents are preferred to directional vents.

W. FIRE PROTECTION

1. If required, laboratory may have a sprinkler system.

2. Sprinkler escutcheon plates must be sealed.

SUMMARY OF BIOSAFETY LEVELS

Facility Biosafety Level		Practices & Safety Techniques	Facilities Equipment
1	Standard microbiological practices.	Containment provided by adherence to standard laboratory practices during open bench operations.	None
2	Level 1 practices plus: Laboratory coats; decontamination of all infectious wastes; limited access; protective gloves and biohazard warning signs as indicated.	Partial containment equipment,(i.e., Class II biological safety cabinets) used to conduct mechanical and manipulative procedures that have high aerosol potential that may increase the risk of exposure to personnel.	Basic
3	Level 2 practices plus: special laboratory clothing; controlled access.	Partial containment equipment used for all manipulations of infectious material.	Containment
4	Level 3 practices plus: entrance through changeroom where street clothing is removed and laboratory clothing is put on; shower on exit; all wastes are decontaminated on exit from the facility.	Maximum containment equipment (Class III biological safety cabinet or partial containment equipment in combination with full-body, air-supplied, positive-pressure personnel suit used for all activities.	Maximum Containment

CLASSIFICATION OF ETIOLOGIC AGENTS ON THE BASIS OF HAZARD

The CDC originally prepared a document which provided a standard for evaluating the hazards associated with the various bacterial, fungal, parasitic, and viral etiologic agents.[1] The list of agents was revised by the NIH for the Recombinant DNA guidelines. A more recent document describes biosafety precautions for use of agents which have resulted in known laboratory infections.[2]

In the CDC documents, human etiologic agents were placed in classes of increasing hazard. The classification is included here as a general reference. Specific examples of agents are listed under each class.

A. *Class 1* Agents of no or minimal hazard under ordinary conditions of handling at Biosafety Level 1.

B. *Class 2* Agents of ordinary potential hazard. This class includes agents which may produce disease of varying degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by the ordinary laboratory techniques described in Biosafety Level 2.

C. *Class 3* Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment, usually at Biosafety Level 3.

D. *Class 4* Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area. Biosafety Level 4 facilities are limited to places such as the CDC, NIH, etc. No Biosafety Level 4 work is permitted on campus. Collaboration may be arranged with United States Army Medical Research Institute of Infectious Diseases or the Frederick Cancer Research Center in nearby Frederick, MD.

E. *Class 5* Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy. Note: Federally licensed vaccines containing live bacteria or viruses are not subject to these classifications. These classifications are applicable, however, to cultures of the strains used for the vaccine production, or further passages of the vaccine strains. This classification does not include strictly animal pathogens. A PHS permit is

required to import any agent or to transfer within the United States any agent imported under permit. Containment conditions are established by the USDA on a case-by-case basis. No Biosafety Level 4 work is permitted on campus.

CLASSIFICATION OF ONCOGENIC VIRUSES ON THE BASIS OF HAZARD

The National Cancer Institute (NCI) classified oncogenic viruses into three classes according to level of risk.[5] Criteria have been developed to identify oncogenic viruses of moderate and high risk to man. All other oncogenic viruses are considered low risk. The criteria are not absolute and are subject to modification as research continues.

A. CRITERIA FOR MODERATE RISK ONCOGENIC VIRUSES

1. Suspected oncogenic virus isolate from man.

2. Virus that transforms human cells in vitro, as evidenced by a morphological and/or functional alteration that is transferred genetically.

3. Virus that produces cancer with the aid of experimental host modification in either a subhuman primate at any age or across mammalian species barrier in juvenile or adult animals.
4. A genetic recombinant between an animal oncogenic virus and a microorganism infectious for man shall be considered moderate risk until its oncogenic potential for man is determined.

5. Any concentrated oncogenic virus or infectious transforming viral nucleic acid.

B. CRITERIA FOR HIGH RISK ONCOGENIC VIRUSES

A virus proved to induce cancer in man shall be classified as high risk until its complete hazard potential can be determined.

GENERAL LABORATORY PRACTICES AND PROCEDURES

Routine safety procedures observed in laboratories do help control biohazards. However, there are additional precautions and procedures that must be followed when dealing with known or potentially biohazardous agents. The following paragraphs summarize general safety procedures for biohazard control.

A. EMPLOYEE ACTION

1. Learn the location and operation of fire extinguishers, safety showers, and other safety items in the work area.

2. Learn what actions are to be taken in the event of a spill or accidental exposure; know the location of a stock solution of disinfectant and the procedure for clean-up of biohazardous spills. Keep fresh disinfectant available at the proper dilution.

3. Notify the responsible investigator or supervisor in case of an accident, injury, exposure, or illness.

B. FOOD, DRINK, AND COSMETICS

1. Food, candy, gum, or beverages for human consumption should not be taken into or consumed in any area where work with biohazardous materials is conducted.

2. Refrigerators, freezers, and cold rooms in laboratories where work with biohazardous materials is conducted should not be used for storage of lunches or other food supplies.

3. Drinking fountains should be the sole source of water for drinking by laboratory personnel.

4. Employees should not wear potentially contaminated laboratory clothing into lunchrooms, cafeterias, or designated eating areas.

5. Applying cosmetics, including hand lotion and lip balm, are prohibited in laboratories.

C. HAND WASHING

1. Hands must be washed throughout the day, at intervals dictated by the nature of the work. The hand washing procedure should include at least a 10 second wash with mild soap and water and thorough rinsing.

2. Hands should be washed promptly after removing protective gloves or soiled protective clothing, before leaving the laboratory area, before eating, and before applying cosmetics.

D. BEARDS AND LONG HAIR

Wearing a beard in biohazardous areas is discouraged; it may become involved in transmission of infectious agents. When the work requires the use of face masks or respirators, a proper facial fit cannot be obtained without a clean shaven face. Individuals with long hair are encouraged to wear a suitable hairnet or head cover.

E. BOOKS AND JOURNALS

1. Every effort should be made to limit the number of books and journals kept in biohazardous areas. Books and journals should not be taken into laboratory rooms where biohazardous agents are being used.

2. Books and journals on loan from libraries should not be taken into biohazardous areas.

F. PIPETTES

1. Materials should not be pipetted by mouth. Mechanical pipetting aids must be used for all procedures.

2. Mixtures must not be prepared by bubbling expiratory air through a liquid containing infectious material with a pipette.

3. Infectious material must not be blown out of pipettes.

4. Pipettes used with infectious or toxic materials must be plugged with cotton unless they are being used in a gas tight, Class III biological safety cabinet system.

5. Contaminated pipettes must be placed horizontally in a 15 cm deep, rectangular, stainless steel or polypropylene pan containing enough

disinfectant (such as Wescodyne™ from AMSCO) for complete immersion. The pan and pipettes should be autoclaved as a unit and replaced by a clean pan with fresh disinfectant.

6. Decontaminated disposable pipettes must be discarded into the biohazard box.

G. SYRINGES

1. Avoid unnecessary use of syringes and needles. Whenever possible, use a blunt needle or cannula on the syringe. Do not use a syringe and needle as a substitute for a pipette.

2. Use syringes and needles in a biological safety cabinet if possible.

3. Use syringes of the LUER-LOK type to assure that the needle cannot separate during use.

4. Use an alcohol pledget around the stopper and needle when removing a syringe and needle from a rubber stoppered vaccine bottle.

5. Expel excess fluid and bubbles from syringes vertically into a cotton pledget soaked with disinfectant, or into a small bottle containing disinfectant-soaked cotton.

6. Swab the site of injection with an appropriate antiseptic before and after injection of an animal.

7. Submerge contaminated, non-disposable glass syringes (with attached needles) in a container of disinfectant fluid (such as Wescodyne) in a biological safety cabinet prior to removal for autoclaving. To minimize accidental injection of infectious material, needles should remain on syringes until after autoclaving. When possible, reusable syringes with attached needles should be placed in a stainless steel pan separate from that holding materials to be discarded after autoclaving.

DISPOSAL OF RESEARCH LABORATORY WASTE

A. DEFINITION OF "SHARPS"

"Sharps" are defined by the State of Maryland as; syringes with needles, needles alone, capped needles, scalpels, razor blades, pasteur pipettes, small glass tubes, capillary tubes, butterfly needles, glass pipettes, microscope slides, coverslips, microtome knives, and any other items which can penetrate the skin.

B. DISPOSAL OF LABORATORY WASTE

1. Sharps must never be placed directly into plastic trash bags. Sharps must be discarded into an approved sharps container.

2. Needles must never be left on laboratory furniture, wrapped in paper towels, or covered by other materials.

3. Needles must never be clipped or bent.

4. Needles must not be recapped using both hands.

5. Laboratory waste must never be discarded directly into the general trash or into red bags. All lab waste must be discarded into the red bag-lined biohazard box.

6. Only approved containers are acceptable. Do not substitute glass, metal or plastic jars, bottles or cans.

7. A broken glass box with a red bag liner is not an acceptable substitute for a red bag-lined biohazard box.

C. APPROVED LABORATORY WASTE CONTAINERS

Containers approved for laboratory waste can be purchased from The Johns Hopkins Hospital Central Stores and The Johns Hopkins University Supply Rooms:

1. Six-quart plastic sharps disposal container with stand:

University - (JHU Cat. # 504491);
(Metal Stand JHU Cat. # 504501)

Hospital - (JHH Container Cat. # 0304310)

2. Ten-quart plastic sharps disposal container with stand:

   Hospital Only - (JHH Cat. # 0304300) same style

3. Cardboard biohazard box with red plastic liner:

   University - (JHU Biohazard Box Cat. # 504591)

   Hospital - (JHH Biohazard Box Cat. # 03040350)

4. University orange autoclave bags (for autoclave decontamination only)

   University - (JHU Cat. # 504272)

D. UTILIZATION OF SIX OR TEN QUART PLASTIC CONTAINERS

1. The plastic sharps disposal containers are used for discarding needles attached to syringes or cartridges, needles, small glassware and other sharps (as defined above) at the point of use. The larger, ten-quart plastic sharps disposal container is available for disposal of higher volumes of sharps, including pasteur pipettes and larger-sized sharps.

2. Before use, the plastic sharps containers must be put in the metal stand designed for the container. The screw cap cover is to be left off until the container is ready for disposal.

3. When discarded sharps reach the fill level designated on the sharps container (at the constriction), screw on the cap, remove the closed container from the metal stand. (Handle sharps containers with caution, they are puncture resistant, not puncture proof). Discard the closed container into a red bag-lined biohazard box.

E. UTILIZATION OF THE BIOHAZARD BOX

1. The cardboard biohazard box with red plastic liner is for disposal of all laboratory waste; including, but not limited to, sharps containers, pipettes, autoclaved waste material, blood tubes, materials soiled with potentially infectious agents,

blood, tissue, or body fluids, calibrated plastic centrifuge tubes, conical tubes and pipettes, glass, and paper towels.

2. All solid, autoclave-decontaminated materials must be discarded into the biohazard box.

3. Each biohazard box used for disposal of laboratory waste must contain a red bag liner at least 3 mil thick or equivalent.

4. The red bag liner must not be removed from the box.

5. The biohazard box cannot be reused.

6. When the biohazard box is full, the red bag liner should be carefully closed and sealed with tape. Then, the box top should be closed, locked with the two tabs and sealed with tape. Custodial Services will take the box away.

7. Pasteur pipettes, needles and syringes, and similar "sharps" contaminated with cultures should be placed into an orange, polypropylene autoclave bag-lined, stainless steel or polypropylene pan with cover containing enough disinfectant solution, such as Wescodyne (diluted 1:10), to cover the pipettes, and then autoclaved. Autoclaved pasteur pipettes, needles and syringes are then discarded into the biohazard box.

F. ADDITIONAL CONTAINERS

Requests for approval of additional containers should be made through the Office of Safety & Environmental Health.

G. SAFE DISPOSAL OF SHARPS CONTAINERS

1. Handle all sharps containers with caution, they are puncture resistant, not puncture proof.

2. Filled containers must be closed as appropriate and replaced.

3. All filled, plastic sharps containers are considered to be infectious waste even if filled with non-infectious materials.

4. All properly closed plastic sharps containers must be placed in an approved biohazard box.

5. All plastic sharps containers that have been autoclaved may have "sharps" protruding through the side. The containers must be placed into an approved biohazard box, never directly into a red bag.

H. DISPOSAL OF GENERAL LABORATORY GLASS

All laboratory glass, including reagent bottles and similar materials, must be rinsed and discarded into the biohazard box.

I. SUMMARY OF WASTE DISPOSAL

1. All laboratory waste must be discarded into Office of Safety and Environmental Health (OSEH) approved red bag-lined biohazard boxes.

2. Cultures of bacteria, fungi, viruses, other microorganisms, and insects must be autoclaved before discarding into a biohazard box.

3. There is one approved red bag-lined biohazard box:

   - JHU Catalog number 504591 biohazard box with red bag liner; and

   - JHH Catalog number 03040350 biohazard box with red bag liner.

4. Call OSEH at 955-5918 for approval to use another biohazard box or to get answers to questions about laboratory waste disposal.

5. All sharps (defined as any material capable of piercing skin, including but not limited to needles, razor blades, pasteur pipettes, and capillary tubes (must be

discarded into the OSEH approved sharps container (JHU Catalog number 504491; or JHH Catalog number 0304310)

6. Glass, metal or plastic jars, bottles and cans are not acceptable substitutes for the approved plastic sharps container.

7. Filled sharps containers must be sealed and discarded into the OSEH approved red bag-lined biohazard box.

8. Broken glass boxes are not acceptable substitutes for biohazard boxes.

9. Do not, under any circumstances, put laboratory waste outside the building.

10. Call Custodial Services for pickup of filled biohazard boxes.

CENTRIFUGE CONTAINMENT

A. CENTRIFUGE PRECAUTIONS

When used properly, centrifuges should not produce aerosols. However, an accident resulting in breakage of a centrifuge tube or bottle can result in a massive exposure. Centrifuges should not be used in corridors or other unauthorized locations.

Certain precautions should be considered when using free standing centrifuges:

1. A swinging bucket head should be used in preference to a fixed angle head because there is a lower probability of aerosol generation.

2. Centrifuge models should be chosen which have a sealed rotor, or sealed buckets, or with at least a guard bowl and gasketed cover. Safety centrifuge cups (tube or bottle carrier with sealable cap or "O" gasketed cap) should be used.

3. The centrifuge chamber should be fitted with a HEPA-filtered exhaust system to capture and remove aerosols from the chamber when containment is needed.

4. Centrifuge tubes or bottles should only be filled, loaded into rotors, and removed from rotors from within a biological safety cabinet. This practice provides containment in case a tube or bottle leaks or breaks.

B. CENTRIFUGE TUBES

1. Before use, tubes should be checked for cracks. The inside of trunnion cups should be inspected for rough walls caused by erosion or adhering matter, and glass pieces should be carefully removed from the rubber cushion. A germicidal solution added between the tube and trunnion cup not only disinfects the outer surfaces of both, but also provides a cushion against shocks that might otherwise break the tube. Metal or plastic tubes (other than nitrocellulose) should be used whenever possible.

2. Never overfill a centrifuge tube or bottle, particularly when using a fixed angle head. Avoid filling tubes to the point where the rim of the centrifuge head becomes wet during a run.

3. Decanting from centrifuge tubes should be avoided. If decanting is necessary, the outer rim should be wiped with a disinfectant.

4. If a tube breaks, the centrifuge should be turned off, allowed to stand undisturbed for 15 minutes, opened, and disinfected. The rotor should also be disinfected.

5. After use, tubes, rotors, and centrifuge interiors should be adequately cleaned and disinfected.

C. HIGH-SPEED CENTRIFUGES

1. High-speed centrifuge chambers are connected to a vacuum pump. If there is a breakage or accidental dispersion of infected particles, the pump and pump oil will become contaminated. A HEPA filter should be placed between the centrifuge inner chamber and the vacuum pump when containment is needed.

2. High speed rotor heads are prone to metal fatigue. Each rotor should be accompanied by its own log book indicating the number of hours run at top or de-rated speeds. Failure to observe this precaution can result in dangerous and expensive rotor disintegration. Frequent inspections, cleaning and drying are important to ensure absence of corrosion or other traumata which cause rotor cracks. Rubber O-rings and tube closures must be examined for deterioration and be kept lubricated with material recommended by the

manufacturer (high temperature silicone vacuum grease). Where tubes of different material are provided (e.g., celluloid, polypropylene, stainless steel), make sure that you are using the tube closure that was designed specifically for the tube being used. These caps are often similar in appearance, but are prone to leakage if put on tubes of the wrong material. When properly designed tubes and rotors are well maintained and handled, leakage should not occur.

3. Cleaning and disinfection of tubes, rotors and other components requires considerable care. No single method is suitable for all items, and the various manufacturers' recommendations must be followed meticulously if rotor fatigue, distortion and corrosion are to be avoided.

4. Manufacturers' safety notices and recalls may not reach you if a transfer of owner/user has occurred unless you notify the manufacturer.

D. CONTAINMENT CENTRIFUGATION

Author: Clarence V. Hall, M.P.H., Washington State Department of Social and Health Services, Environmental Laboratory Services Unit.

Although centrifuging infectious material is one of the greatest aerosol producing operations in a microbiology laboratory, there are currently no proven safety devices for centrifugation available on the market. Therefore, at the Washington State Public Health Laboratory, a method was devised to capture and remove aerosols from the air in the centrifuge. The method employs only a few inexpensive parts and is adaptable to any centrifuge having an enclosed chamber with an air intake hole in the lid and exhaust hole in the bottom of the chamber.

In our laboratory we used parts which are listed herein not as an endorsement of the manufacturer or supplier, but as an example of the type of equipment needed. Our basic unit is an International Model UV centrifuge. A High Efficiency Particulate Aerosol (HEPA) filter with three inch outside diameter fittings for both air intake and exhaust was obtained from Flanders, Stock Number 0-007-C-08-N2-IL-00-00-BBD. A Dayton 4C441, 60 cubic feet per minute free air capacity blower was purchased from W.W. Grainger. Two inch inside diameter Autoclavable flexible ducting and clamps were obtained from a Seattle firm, Steam Supply and Rubber Company. A two inch outside diameter short stainless steel flanged nipple and a metal adapter for the blower-duct connection were made at a local sheet metal shop.

Assembly of the parts can be accomplished in the following manner: Fit the flanged nipple into the centrifuge chamber exhaust hole, solder or weld it in place, and grind the joint to provide a smooth surface inside the chamber. Place the HEPA filter on a shelf mounted on the back of the centrifuge or on a nearby wall. Fasten the blower near the HEPA filter and ducting ends.

* Health Lab. Sci. 12, 104-106 (1975). Cutaway drawing showing assembly of entilation parts.

The blower is used to overcome the resistance of air through the filter. It also provides needed air exchanges within the centrifuge chamber after the centrifuge head stops spinning. Air from the blower exhaust should be ducted to the outside.

If breakage occurs during centrifugation, the vented centrifuge can be readily decontaminated. After the centrifuge head stops rotating allow several air changes (we allow at least 30) to occur in the chamber before opening the lid. This should effectively remove aerosol particles. The inside of the chamber and lid and the centrifuge head and shaft should then be wiped down with an appropriate germicide. Rinse or swab the nipple connection and allow the excess germicide and spilled liquids to collect in the downward loop of the flexible ducting. The ducting should then be carefully removed and autoclaved along with the shields and carriers.

The actual air exchanges per minute can be easily calculated and should be determined for each centrifuge venting system. Measure the linear air flow in feet per minute from the exit port of the blower. For this measurement we used a velometer. Compute the area of the exit port in square feet (π^2). Determine the internal volume of the centrifuge chamber in cubic feet (π^2 x height). Calculate according to the formula:

Air Changes per Minute = Internal Chamber Volume Air Flow X Area of Exhaust Port

$$\frac{\text{Air Flow x Area of Exhaust Port}}{\text{Internal Chamber Volume}}$$

With both centrifuge and blower operating, our system provides 38 air changes per minute. The blower alone supplies approximately 10 air changes per minute through the chamber. Therefore, when breakage occurs we stop the centrifuge and wait four minutes before opening the lid.

BLENDERS, SONICATORS, MILLS, GRINDERS & CELL SORTERS

These devices release considerable aerosols during operation. For maximum protection to the operator during blending or mixing of infectious materials, the following practices should be observed.

A. Operate blending, cell-disruption and grinding equipment only in a biological safety cabinet. Place a towel moistened with disinfectant over the top of the blender.

B. Use safety blenders designed to prevent leakage from the rotor bearing at the bottom of the bowl.

C. If you don't have a leak-proof rotor, inspect the rotor bearing at the bottom of the blender bowl for leakage prior to operation. Test it in a preliminary run with sterile saline or methylene blue solution prior to use with infected material.

D. Autoclave the device and residual infectious contents promptly after use.

E. Glass blender bowls are undesirable for use with infectious materials because of potential breakage. If used, they should be covered with a polypropylene jar to prevent dispersal of glass in case of breakage.

F. A heat-sealed flexible plastic film enclosure for a grinder or blender can be used, but it must be opened in a biological safety cabinet.

G. Before opening, permit the blender to stand for at least 15 minutes after the run to allow the aerosol cloud to settle within the chamber.

H. Clinical or other laboratories handling human blood or tissue should be aware of possible biohazardous aerosols produced by microhaematocrit centrifuges, autoanalyzer stirrers, microtonometers, FACS's, etc.

WATER BATH DISINFECTANTS

Water baths and Warburg baths used to inactivate, incubate, or test biohazardous materials should contain a disinfectant. For cold water baths, 70% propylene glycol is recommended. Wescodyne™ (AMSCO) works well in intermediate temperature water baths. Azides should not be used as a bacteriostatic agent because they may form heavy metal azides. These are shock-sensitive and may explode. Change disinfectant frequently.

LABORATORY VACUUM TRAPS

When laboratory vacuum is used to manipulate biohazardous materials, a suitable trap must be employed to insure that building vacuum pump and compressed air lines (they are often linked together in the building mechanical room) do not become contaminated.

Laboratory vacuum service should be provided by the use of small, individual vacuum pumps fitted with high efficiency particulate air (HEPA) filters on the suction side. Central house systems are not recommended. If house vacuum must be used, the system should include in-line HEPA filter as near as practical to each point of use or service cock. An approved reservoir and filtration apparatus for vacuum systems is described below:

Vacuum filtration or aspirating supernatants into collection flasks are common laboratory procedures. During vacuum filtration or aspiration procedures building and/or laboratory vacuum systems should be protected.

A simple bench-top aerosol/fluid trap can protect building / laboratory vacuum systems. The basic vacuum trap consists of a disposable cartridge-type filter (JHU Cat.# 504481) or equivalent installed in-line with a collection/overflow vacuum flask system. OSEH recommends the general set-up pictured below:

The aerosol/fluid trap consists of two vacuum flasks, preferably plastic, (size dependent on amount of fluid that may accidentally be aspirated out of the collection flask), thick walled plastic tubing (to prevent tubing collapse), rubber stoppers, a filter (prevents unwanted potentially biohazardous fluid and aerosols from entering vacuum systems), and a ceramic sparger (ceramic fish tank bubbler) immersed in disinfectant. The sparger disperses aerosols passing out of the collection flask into small bubbles so that adequate contact is made with a disinfectant solution. Use an appropriate disinfectant solution shown to be effective on the biohazardous material under study. It is essential that anti-foam

spray (such as Thomas Scientific Cat.# 1130-D15 or VWR Cat.# JTB531-5) be added to the overflow flask since air bubbling through the disinfectant-immersed sparger may produce foam that could shut off the vacuum if allowed to clog the filter.

When the filter or overflow flask require routine changing, they can be safely removed by clamping the line between the filter and the vacuum source before disconnecting the tubing from the source. The filter and vacuum flask should be decontaminated by autoclaving if they have been in contact with potentially biohazardous material.

REFRIGERATORS, FREEZERS AND DRY ICE CHESTS

Refrigerators, freezers and dry ice chests should be checked, cleaned out, and defrosted periodically to remove any ampules, tubes, etc., containing biohazardous materials that may have broken during storage. They must be discarded into a biohazard box. Rubber gloves are recommended for cleaning. All materials, especially infectious or toxic materials stored in refrigerators or freezers, should be labeled with their scientific name, date stored, and name of the individual storing the material. Do not store flammable solutions in refrigerators unless they are approved by the JHI Occupational Safety Officer. Standard domestic refrigerators must not be used for storage of flammable materials.

When storing biohazardous materials in liquid nitrogen, use gasketed, screw-cap plastic ampules whenever possible because these are specially designed for this purpose and reduce the risk of accidental shattering or leakage. Wear protective gloves and a face shield when working with liquid nitrogen storage equipment.

Before moving, repairing or discarding refrigerators, freezers, and similar equipment contact the Biosafety Officer. The Biosafety Officer will place a "Cleared for Maintenance Work" notice on the equipment for facilities and non-Hopkins employees who handle this equipment.

TRANSPORTATION OF BIOHAZARDOUS MATERIAL

A. Transportation of potentially pathogenic / oncogenic fluid cultures or toxins and viable, powdered biohazardous materials should be transported, incubated, and stored in easily handled, non-breakable, leak-proof pans, trays, pails, carboy holders, or other secondary containers large enough to contain all the fluid or powder in case of leakage or breakage of the primary container. All inoculated petri plates or other inoculated solid media should be transported in leak-proof containers.

Containers of biohazardous materials to be transferred between buildings must be placed inside non-breakable containers having solid sides and bottoms and tight covers to prevent breakage or spillage during transit.

B. Shipment of biohazardous materials such as cultures or biological toxins and/or diagnostic or clinical specimens which the shipper expects may contain etiologic agents (such as bloodborne pathogens) must conform to 42 CFR Part 72.3 "Interstate Shipment of Etiologic Agents", 1980. Packages must contain a secondary container, absorbent material between the primary and secondary container, and an outer shipping container with proper labeling. Contact the JHI Biosafety Officer for current shipping guidelines.

TEST TUBE / VACUTAINER TECHNIQUES

Tubes containing cultures, blood, or other biohazardous materials should be manipulated with extreme care. Studies have shown that a simple procedure such as removing a tube cap or transferring an inoculum can create a potentially hazardous aerosol.

Manipulation of biohazardous material should be conducted in biological safety cabinets. Tubes and racks of tubes containing biohazardous material should be clearly marked. It is the responsibility of the individual employee to insure that tubes containing biohazardous material are properly decontaminated prior to disposal and/or glassware washing. Whenever possible, use test tube trays with a solid bottom and sides deep enough to hold all spillage from broken tubes.

MEMBRANE FILTERS

Care should be exercised during membrane filtration to obtain sterile filtrates of biohazardous materials. Due to the fragility of the membranes and other factors, such filtrates should not be considered noninfectious until culture or other tests have proved their sterility.

HAZARDOUS OPERATIONS (TWO PERSON RULE)

Each principal investigator or supervisor is responsible for insuring that no person works alone on a biohazardous operation, such as a biosafety level 3 facility.

No person shall work alone in a containment facility in a remote location. The containment facility (biosafety levels 3 and 4) must have viewing panels that allow people outside the containment facility to see into the facility.

USE OF DRY POWDERS

The preparation, handling, and use of dry powders of biohazardous materials present hazards of an unusual nature. The slightest manipulation of such powders may release an aerosol containing high concentrations of infectious or toxic material. Therefore, work with dry powders of biohazardous materials should be done only in biological safety cabinets, fume hoods with exhaust duct filters, or in glove boxes with slight negative airflow. Dry powders are difficult to decontaminate, particularly with liquid disinfectants. Disinfectant applications should be thorough and with longer than usual contact times.

LABORATORY CONSTRUCTION AND RENOVATION

A. All construction and/or renovation of facilities to be used for biohazardous agents or materials must be reviewed in the planning stage by the Office of Safety and Environmental Health in cooperation with the Plant Manager's Office, Facilities Design and Construction, and other support groups. This will insure that proper biohazards control design concepts have been included in the proposed design and that all biohazard risk to construction personnel has been eliminated when the project involves an renovation of an existing facility.

B. Facilities Management should arrange through the Office of Safety and Environmental Health an inspection to verify that the area to be renovated is clear of hazards.

ANIMAL CARE AND HANDLING

Special attention should be given to the humane treatment of all laboratory animals in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23) and the Federal Laboratory Animal Welfare Act (Public Law 91579) or later editions. See also *The Use of Experimental Animals at The Johns Hopkins Medical Institutions and University*, obtainable from the Division of Comparative Medicine.

DECONTAMINATION AND DISINFECTION PROCEDURES

A. All infectious or toxic materials, and all contaminated equipment or apparatus should be decontaminated before being washed and stored, or discarded. Autoclaving is the preferred method. Each individual working with biohazardous materials should be responsible for decontamination before disposal.

B. To minimize hazard to firemen or disaster crews, all biohazardous materials should be placed in an appropriately marked refrigerator or incubator, or decontaminated, or otherwise confined at the close of each work day.

C. All autoclaves should be certified for operating efficiency by the frequent use of biological indicator controls. All autoclaves should bear a sign indicating the maximum permissible pressures and last date of certification.

D. Special precautions should be taken to prevent accidental removal of material from an autoclave before it has been sterilized or decontaminated. Simultaneous opening of both doors on a double door autoclave must not occur. Biohazardous

materials should not be placed in autoclaves overnight in anticipation of autoclaving the next day.

E. Dry hypochlorites, **liquid bleach, and any other strong oxidizing material, must NOT be autoclaved**.

F. All laboratory rooms containing biohazardous materials should designate two separate carts or containers labeled;

1. BIOHAZARDOUS - TO BE AUTOCLAVED BEFORE CLEANING

2. NON-INFECTIOUS - TO BE CLEANED

G. All floors, laboratory benches, and other surfaces in buildings where biohazardous materials are handled should be disinfected as often as deemed necessary by the supervisor. The surroundings should be disinfected after completion of operations involving plating, pipetting, centrifuging and similar procedures with biohazardous materials. It is the responsibility of the supervisor to determine that the disinfectant and the time and method of exposure is effective against the biological agent(s) used in the facility.

H. Drains should be flooded with water or disinfectant at least once each week in order to fill traps and thus prevent the backflow of sewer gases. Drains in animal biosafety level 3 facilities must be closed and sealed.

I. Floor cleaning procedures which minimize the generation of environmental aerosols should be used. Wet mopping or wet vacuum pickup is recommended. Water used to mop floors should contain a disinfectant or disinfectant-detergent. (Dry mopping or dusting should be avoided). Where wet procedures are not practicable, dry vacuum cleaning with a HEPA filter on the exhaust, sweeping compound used with push brooms, or dry dust mop heads treated to suppress aerosolization may be used.

J. Stock solutions of suitable disinfectants should be maintained in each laboratory for disinfection purposes. The disinfectant should be kept readily available in the use-dilution.

STEAM AUTOCLAVING

A. Steam sterilization of clean materials is a dependable procedure for the destruction of all forms of microbial life. Steam sterilization generally denotes heating in an autoclave employing saturated steam under a pressure of approximately 15 psi to achieve a chamber temperature of at least 121°C (250°F) for a minimum of 15 minutes. The time is measured after the temperature of the material being sterilized reaches 121°C (250°F). The most critical factor in insuring the reliability of this sterilization method other than proper temperature and time is the prevention of entrapment of air that is not replaced by steam. Some autoclaves utilize a steam activated exhaust valve that remains open during the replacement of air by live steam until the steam triggers the valve to close. Others utilize a pre-cycle vacuum to remove air prior to steam introduction.

B. Physical controls such as pressure gauges and thermometers are widely used but are considered secondary methods of insuring sterilization. The use of appropriate biological indicators at locations throughout the autoclave is considered the best indicator of sterilization. The biological indicator most widely used for wet heat sterilization is a *Bacillus stearothermophilus* spore suspension or strip.

C. If sterilization is associated with patient diagnosis, the biological indicator and associated documentation is required by regulation.

AUTOCLAVE DECONTAMINATION PROCEDURES

This establishes guidelines for the effective use of steam autoclaves for the decontamination of cultures and other potentially pathogenic materials and protects personnel and the environment from these potentially infectious materials and waste. This will also meet the waste disposal requirements of the State of Maryland, AAMI, AHA, CDC, JCAHO, and JHH/JHU.

A. BACKGROUND

The following basic guidelines should help you develop your own departmental autoclave quality assurance program for monitoring autoclave decontamination effectiveness. Effective quality assurance includes: using chemical and biological indicators to check autoclave operation; selecting appropriate containers to hold waste while being decontaminated; using effective decontamination processing times for each load; maintaining proper autoclave use records; providing odor control when necessary; and providing personnel training for the operation of an autoclave.

Use of steam sterilizers (autoclaves) should include validation of decontamination effectiveness. Validation of effectiveness includes monitoring temperature, pressure and cycle duration time for each cycle and providing periodic decontamination challenges (quality assurance), i.e. use of biological indicators. A logbook should be maintained to record autoclave use. The logbook should be available for inspection by various agencies and authorities.

B. <u>INDICATORS</u>

1. CHEMICAL INDICATORS

a. Chemical Color Change Indicators:

Chemical indicators for steam autoclaves change color after being exposed for a few minutes to normal autoclave operating temperatures of 121°C (250°F). Hence, chemical indicators can give you a quick visual reference for heat penetration inside the load. Chemical indicators should be positioned near the center of each load, and toward the bottom front of the autoclave.

CAUTION: Most chemical indicators can only be used to verify that your autoclave has reached normal operating temperature for decontamination, 121°C (250°F); they have no time factor. Chemical indicators alone are not designed nor intended to prove that organisms are actually killed during a decontamination cycle.

Where to Purchase Chemical Indicators: Chemical indicators are manufactured by many companies and come in a wide variety of sizes, shapes, and colors. Some examples are: "Chemdi Strips" - Amsco, "Dualchek and Single Indicators" - Baxter, "Test-A-Clave" - Baxter, "ATI Indicator Tape" - Thomas Scientific, "ATI Sterilometer-Plus 250" - Thomas Scientific, "Sterikon-Bioindicator" from EM - VWR, and "TSI Time Indicator" - VWR.

b. Tape Indicators:

Tape indicators are adhesive backed paper tape with heat sensitive, chemical indicator markings. Commonly used heat sensitive markings include diagonal stripes (autoclave tape), and/or the word "sterile". These markings only appear when the tape has been

exposed for a few minutes to normal autoclave decontamination temperatures.

CAUTION: Tape indicators can only be used to verify that the autoclave is reaching normal operating temperatures for decontamination, 121°C (250°F). Tape indicators alone are not designed nor intended to prove that organisms have actually been killed.

Tape indicators should be used on all material decontaminated by autoclaving to show that the material has been processed. A three to four inch strip of autoclave tape placed on the outside of the autoclave pan, bag, or individual container is sufficient.

2. BIOLOGICAL INDICATORS

Biological indicator systems are designed to demonstrate that an autoclave is capable of killing microorganisms. Only Bacillus stearothermophilus spores can be used to monitor the effectiveness of steam autoclaves.

Typical biological indicator systems consist of a vial with spore strips or a small glass ampule of growth medium with spores and indicator dye. Refer to manufacturers' instructions for use. The biological indicator is removed from a load after it has been autoclaved. Then the biological indicator is incubated at 56°C for up to three days. Your control vial, which was not autoclaved, should be turbid after incubation; the successfully decontaminated test vial should remain clear without evidence of turbidity (no growth). If the autoclaved biological indicator is turbid (cloudy, indicating growth) the autoclave did not function properly. Notify your area supervisor when this happens.

Where to Purchase Biological Indicators: Some manufacturers of biological indicators have incubators available to fit their vials. Some examples of biological indicators are: "Spordex Sprore Strips or Suspensions and "Proof Systems" - Amsco, "Tower Spore" - Baxter and "Spore Strips" from Ravin - Baxter or VWR.

C. CONTAINERS

Materials that are to be decontaminated should be carried to the autoclave in closed, leak proof containers. Containers used to hold material while being autoclaved are described below.

1. PRIMARY CONTAINERS

Plastic Autoclave Bags:

Polypropylene bags are used to contain materials during decontamination cycles within autoclaves. Also known as "biohazard bags" or "autoclavable bags", autoclave bags come in a wide variety of sizes, shapes and colors. The JHI- recommended orange polypropylene autoclave bag is available at the University Supply Store (Cat. # 504272).

Autoclave bags are usually placed in polypropylene or stainless steel pans during decontamination cycles to catch liquids that may drain out of the bag. Autoclave bags should be left open during decontamination to allow steam to penetrate into the bag. Additional water should be added to the bag's interior to facilitate heat transfer to the items being decontaminated. The amount of extra water added to the bag should be determined experimentally by the autoclave user, based upon the type and size of material being decontaminated. Do not add water if there is a chance that potentially infectious materials may splash out of the bag.

Autoclave bags being filled in a laboratory should be temporarily placed inside a red bag-lined biohazard box. The lab personnel can then take the autoclave bag and biohazard box combination to the autoclave. The autoclave bag should be removed from the box, decontaminated in the autoclave and discarded into the red bag lined biohazard box.

2. SECONDARY CONTAINERS

a. Plastic Containers:

Polypropylene is a durable, inexpensive plastic resin which is commonly used contain material during autoclaving. Polypropylene plastic pans with 6-12 inch sides are favored over polyethylene and polystyrene because polypropylene can withstand autoclaving without melting.

Note: When using polypropylene containers, add extra processing time to the autoclave decontamination cycle because polypropylene does not conduct heat as well as stainless steel.

b. Stainless Steel Containers:

Stainless steel containers are durable and come in a variety of sizes and shapes. Because stainless steel is a good conductor of heat, autoclave decontamination cycle processing time may be reduced. Where waste containment is mandatory, stainless steel containers may be the container of choice because it is durable. Restaurant supply companies are a good source for these pans.

D. AUTOCLAVE OPERATION

1. CAUTION: Only personnel with adequate training on autoclave use should be permitted to operate an autoclave. Personnel should wear proper personal protective equipment, ie. heat resistant gloves, eye protection, etc. particularly when unloading the autoclave.

2. Regularly inspect your autoclave components for proper operation. Autoclave door clamps and seals should be inspected for wear and damage. Also remove debris from the autoclave chamber floor drain. If a problem is found, promptly notify your area supervisor who will call facilities or maintenance. DO NOT OPERATE AN AUTOCLAVE UNTIL IT HAS BEEN PROPERLY REPAIRED.

3. At the end of a decontamination cycle make sure that the pressure in the autoclave chamber is near zero before opening the door. Slowly crack open the autoclave door and allow the steam to gradually escape from within the autoclave.

CAUTION: Opening the autoclave door too quickly may result in glassware breakage and/or steam burns on your skin.

Allow materials inside the autoclave to cool for 10 minutes before removing them from the autoclave.

Avoid dead air pockets where steam cannot penetrate (ie., closed screw cap tubes) because temperature within the air pocket may be lower than the saturated steam.

Avoid dry packages, add some water to the load. To avoid creation of infectious aerosols while adding water, trickle water down the sides of the container instead of pouring water directly onto the material in the container or bag.

E. PROCESSING TIMES

1. After loading and starting the autoclave, processing time starts after the autoclave reaches normal operating conditions: Temperature = 121°C (250°F); Pressure = 15 psi.

2. Decontamination conditions vary with type of load, load volume (loose packed or tightly packed), container type (polypropylene, glass, stainless steel), and type of material to be decontaminated. Therefore, processing times will vary according to the conditions of each decontamination cycle. In general, the larger the load, the longer the decontamination time.

3. Sixty minutes are needed to decontaminate lab and medical waste, unless a shorter interval has proven effective by testing with biological indicators. Add additional time if polypropylene containers are used instead of stainless steel containers.

4. Ninety minutes are recommended for decontamination of waste in low sided polypropylene containers with bags half filled and loosely gathered. If bags are tightly closed, a processing time of 120 minutes may be needed.

5. If your autoclave is equipped to operate at 132°C (270°F), you may be able to reduce processing time if biological indicator spores placed in the center of the material to be decontaminated are killed during shorter autoclaving times.

6. The US Environmental Protection Agency (EPA) has reported that....."Infectious wastes from departments of health care facilities may be rendered noninfectious by subjecting the waste to autoclave temperatures of 121°C (250°F) and 15 minutes of prevacuum of 15 psi for the following dwell times when proper containers are used:"

EPA Recommended Decontamination Processing (Dwell) Times

TRASH	60 Minutes
GLASSWARE	60 Minutes
LIQUIDS	60 Minutes/ Gallon
ANIMAL CARCASSES	8 Hours
ANIMAL BEDDING	8 Hours

F. ODOR CONTROL

Some waste material has an extremely noxious odor, i.e. anaerobic bacteria, feces or decaying organic materials. When decontaminating these materials, it may become necessary to add an odor control additive to the load. A few examples of odor control additives are: any absorbent kitty litter, "Odo-Clave", "Decon Decap" - Thomas Scientific.

G. TRAINING

Principal investigators, directors or supervisors must train and qualify their staff for operation of steam autoclaves for decontamination of materials. Qualified autoclave users should understand the time, temperature, pressure relationships required for proper materials decontamination. Additional training on handling materials to be decontaminated should also be provided. Supervisors should maintain a permanent record of training provided to their staff.

Additional training support is available by contacting the Biosafety Officer.

H. RECORD KEEPING

1. A durable notebook should be used as a permanent record of autoclave use. The autoclave log book should be located in an easily accessed location

near the autoclave. The autoclave log book should have at least the following information entered;

Autoclave Manufacturer,
Autoclave Serial Number,
Department,
Autoclave Room Location,
Date Log Book Started,
Maintenance Work Done, and
Materials Processed.

2. The main section of the autoclave record should include;

Autoclave User,
Date Used,
Materials Decontaminated,
Process Type,
Run Duration (Cycle Time),
Chemical/Biological Indicator Used,
Chemical/Biological Indicator Results, and
Envelope for "Wheel Graphs" or "Data Strips".

I. AUTOCLAVE LOG BOOK SAMPLES

Samples of autoclave process information sheets for your logbook are attached. Make extra copies as needed.

TEMPERATURE CHART

(this can be replaced by saving the paper tape from the autoclave)

ROOM # _______________

AUTOCLAVE # _____________

DATE	RUN #	MEDIA	STERILE ITEMS	TIME	TEMP	NAME

INSTRUMENT MAINTENANCE SCHEDULE

MAINTENANCE BY:

__LAB STAFF _ELEC. TECH

DIVISION: ________ LABORATORY _____

__ENGINEERING _OUTSIDE VENDOR

INSTRUMENT MAINTENANCE: __WEEKLY __4 WEEKS __8 WEEKS __6 MONTHS __YEARLY __EMERGENCY

DATE	SIGNATURE	DATE	SIGNATURE	DATE	SIGNATURE

AUTOCLAVE SERVICE OR REPAIR

ROOM #___________

AUTOCLAVE #_________

REPORTED PROBLEM	DAT E	TECH	CORRECTIVE ACTION	DAT E	DOW N TIME	BY

BIOLOGICAL STERILIZATION CHECK

ROOM #____________

AUTOCLAVE #__________

DATE	RESULTS	SIGNATURE	DATE	RESULTS	SIGNATURE

DRY HEAT STERILIZATION

Dry heat sterilization is less efficient than steam sterilization and requires more time and/or higher temperatures. The specific times and temperatures must be determined for each type of material being sterilized. Generous safety factors are usually added to allow for the variables that can influence the efficiency of this method of sterilization. The moisture of the sterilization environment as well as the moisture history of organisms prior to heat exposure appear to affect the efficiency of dry heat sterilization.

Sterilization of clean materials by dry heat can usually be accomplished at 160-170°C (320-338°C) for periods of 2-4 hours. Higher temperatures and shorter times may be used for heat-resistant materials. The heat transfer properties and the arrangement of articles in the load are critical to insuring effective sterilization.

ETHYLENE OXIDE STERILIZATION

A. Ethylene Oxide (EtO) gas is lethal for microorganisms, including spores, viruses, molds, pathogenic fungi and highly resistant thermophilic bacteria.

B. **EtO must not be used to decontaminate material. It is a surface active only disinfectant and will not penetrate laboratory waste.**

FORMALDEHYDE GAS DECONTAMINATION OF EQUIPMENT

A. INTRODUCTION

1. Decontamination is mandatory when maintenance work, filter changes and performance tests require access to any contaminated or potentially contaminated portion of a BSC. There are some situations when BSC's are decontaminated before performing certification tests, particularly if they have been used with infectious organisms, oncogenic viruses, or etiologic agents in a biosafety level 3 facility. Decontamination generally uses formaldehyde gas. Please contact the Biosafety Officer to arrange for formaldehyde decontamination.

2. BSC's should be decontaminated;

   a. Before moving to a new location,

   b. Before BSC HEPA filters are removed and replaced,

   c. Before internal BSC maintenance is performed,

   d. When the BSC is contaminated from a spill or splash of concentrated biohazardous research material, and

   e. When requested by the Biosafety Officer.

3. Formaldehyde decontamination of laboratory equipment, i.e., biological safety cabinets, centrifuges, incubators, etc., must be conducted by OSEH-authorized professionals. Our professional contractor will supply you and the Biosafety Officer with a written report describing the decontamination technique used. The report should be maintained in your file for future reference because maintenance and service companies responsible for moving and reinstalling your BSC may require documentation that your BSC does not pose a threat to their health and safety. In emergency situations, the Biosafety Officer can provide advice and instructions.

ULTRAVIOLET LIGHT DECONTAMINATION

Under certain conditions of radiation intensity, exposure time, humidity, and temperature, ultraviolet radiation at approximately 254 nanometers will cause eventual death of microorganisms. The radiation at this wavelength causes formation of thymine-thymine dimers and other effects on DNA and RNA. Nucleic acid containing thymine dimers does not replicate properly and lethal mutations are often produced. Ultraviolet light's greatest effectiveness is against actively growing bacteria. Low pressure mercury vapor lamps usually supplied with biological safety cabinets emit germicidal radiation at a wavelength of 254 nanometers for about nine months. After this time, the lamp may not produce enough germicidal radiation to effectively kill bacteria, even though it appears to be functioning properly.

In general, ultraviolet radiation is used to reduce exogenous contaminants and/or pathogenic microorganisms on exposed surfaces and in the air.

A. UV LAMP OPERATION

1. All UV installations used for disinfection should be checked semi-annually. Periodic examination is necessary because UV bulbs may continue to burn without emitting effective radiation. UV lamps should be replaced when they emit 70 percent or less of their rated initial output.

2. UV lamps installed in biological safety cabinets must be replaced when the 254 nm UV irradiation intensity on the work tray surface of the cabinet is less than 40 microwatts per square centimeter.

3. UV lamps should be cleaned often if located in an unusually dusty area. Lamps should be turned off and wiped with a soft pad moistened with alcohol. Cleaning is the responsibility of the personnel in charge of the laboratory.

4. All exposed UV installations in lighting fixtures and safety cabinets shall be turned on **only when no personnel** are in the area. Louvered, wall mounted UV equipment may be left on continuously.

5. Each UV installation should be equipped with an outside switch and an appropriate safety sign. Interlocks should be installed where appropriate to turn off UV lamps when room lights are turned on.

6. Biological safety cabinets listed by the National Sanitation Foundation (NSF) after 1992 may not have UV lamps installed because there is no longer a NSF secondary test standard for UV lamps. Annual testing is required at JHI, however, for BSC's containing UV lamps.

B. TRAINING

All personnel should be instructed in the proper use of each UV installation. Such instruction should include emphasis on the following:

1. Do not look directly at UV lamps;

2. Do not loiter in UV airlocks and door barriers;

3. Turn off lamps before cleaning;

4. Wear eye and skin protection if anticipated exposure to UV will be for longer than a few seconds;

5. Protective goggles should transmit less than 4% of 400 nm wavelength light;

6. Particular care needs to be exercised around UV gel transilluminators, they produce considerable radiation.

CHEMICAL DISINFECTANTS

A. PHENOLIC COMPOUNDS

1. Recommended for the killing of vegetative bacteria, including *Mycobacterium tuberculosis*, fungi and lipid-containing viruses using a concentration of 0.5-2.0%. They are less effective against spores and non-lipid containing viruses.

2. Low solubility in water unless combined with detergent.

3. Stable in storage.

4. Germicidal against Gram-negative and Gram-positive organisms and tubercle bacilli.

5. Less adversely affected by organic matter than other common germicides.

6. Effective over relatively large pH range.

7. Limited sporicidal activity.

8. Prolonged contact deteriorates rubber.

9. Can cause skin and eye irritation.

10. Not for use on food contact surfaces.

B. QUATERNARY AMMONIUM COMPOUNDS

1. Acceptable as general use disinfectants to control vegetative bacteria and non-lipid-containing viruses. However, they are not active against bacterial spores or *Mycobacteria* at the usual use concentrations (1:750).

2. Stable in storage.

3. No odor but act as deodorizers.

4. Use dilution usually non-irritating to skin but prolonged skin or eye contact should be avoided.

5. Effective at temperatures up to 212°F.

6. Effective against Gram-positive organisms, bacteriostatic in high dilutions.

7. Generally ineffective against tubercle bacilli, spores, and viruses.

8. More effective in alkaline than acid solutions.

9. Neutralized by soap and anionic detergents.

10. Effectiveness reduced by organic material.

11. Has built-in detergent properties.

12. Some are active against lipophilic viruses.

C. IODOPHORS

1. Although these show poor activity against bacterial spores, they are recommended for general use in concentrations of 70 to 150 ppm. They are effective against vegetative bacteria and viruses. Wescodyne (Amsco HNJ138) is effective against *M. tuberculosis* when used at the proper dilution.

2. Combine iodine with non-ionic detergent.

3. Rapid biocidal action.

4. Effective against Gram-negative and Gram-positive organisms, some viruses, and tubercle bacilli.

5. Most effective in acid solutions.

6. Vaporize at 120°F to 125°F -- should not be used in hot water.

7. Effectiveness reduced by organic matter (but not as greatly as hypochlorites.)

8. Stable in storage if kept cool and tightly covered.

9. Iodophors are relatively harmless to man.

10. Iodophors have a built-in indicator. If the solution is brown or yellow, it is still active.

11. Iodophors can be readily inactivated and iodophor stains can be readily removed with solutions of sodium thiosulfate.

12. May tarnish silver, silver plate, and copper.

D. ALCOHOLS

1. In concentrations of 70%, alcoholic solutions are good general-use disinfectants, but they exhibit no activity against some bacterial and fungal spores or tubercle bacilli.

2. Germicidal against a broad spectrum of bacterial species and many viruses.

3. Fast acting.

4. Leave no residue.

5. Compatibly combined with other disinfectants (quaternaries, phenolics, and iodine) to form tinctures, extends alcohol's cidal action.

6. Results from experiments conducted at NIH indicate that a combination of 60% ethanol with 0.01N HCl (pH 4) had remarkably improved cidal action against poliovirus and adenovirus.

7. Flammable, not to be used near a flame. Therefore, use of alcohol inside a biological safety cabinet may be a fire safety hazard.

E. ALDEHYDES

1. Effective against wide spectrum of bacteria and viruses. Sporicidal when used properly (10 hour contact period).

2. Formaldehyde Solutions - At concentrations of 8%, formalin exhibits good activity against vegetative bacteria, spores, and viruses. Its use must be limited and controlled because of its toxic properties.

3. Formaldehyde-Alcohol - Solutions of 8% formalin in 70% alcohol are considered very good for disinfection purposes because of their effectiveness against vegetative bacteria, spores and viruses. For some applications, this may be the disinfectant of choice.

4. Activated Glutaraldehyde - Two percent solutions exhibit good activity against vegetative bacteria, spores and viruses. Its use, however, must be limited and controlled because of its toxic properties and potential damage to eyes. Glutaraldehyde must only be used in ventillated hoods. Contact the Environmental Safety Officer if you want to use it for specific applications. It has limited stability after activation (for alkaline glutaraldehyde).

F. CHLORINE COMPOUNDS

1. Recommended for certain disinfecting procedures such as cleanup of blood or body fluid spills when household liquid chlorine bleach is diluted 1:10 with tap water.

2. A 1:10 dilution (5,000 ppm) of bleach has a biocidal effect on *M. tuberculosis*, *S. aureus*, other vegetative bacteria, and HIV after 10-20 minutes.

3. For bacterial spores and mycobacteria, higher concentrations of at least 2,500 ppm (1:5 dilution) are needed.

4. Their decay rates and lack of residuals is such that they must be made up fresh. A 1:50 dilution of chlorine bleach stored at room temperature in a closed plastic container will deteriorate to the equivalent of a 1:100 dilution (500 ppm) after one month, if the container is kept closed (Rutola,W.A., *Am. J. Infect Control.* 1989;17:1).

5. Neutralized rapidly in the presence of organic matter.

6. A 0.5 percent sodium hypochlorite solution (1 to 10 dilution of liquid laundry bleach) is recommended for decontamination of HBV, HIV, and cleanup of biohazardous spills.

7. A fresh solution of 0.05 percent hypochlorite solution (1 to 100 dilution of 5% chlorine liquid laundry bleach) is recommended for general disinfection of surfaces and liquid waste.

    NOTE: FOR CREUTZFELDT-JAKOB DISEASE (CJD) INFECTED TISSUE: Undiluted bleach has been used for surface disinfection after possible contamination with the CJD virus; however, 1.0 N NaOH is currently recommended by the investigators at NIH (See Brown, Wolff, and Gajdusek, *A simple and effective method for inactivating virus infectivity in formalin-fixed tissue samples from patients with Creutzfeldt-Jakob disease*, Neurology **40:** 887-889, 1990.

G. MERCURIALS

1. Not recommended for general use; they have poor activity against vegetative bacteria and are not effective on spores.

2. Although mercurials exhibit good activity against viruses (1:500 to 1:1000 concentration), they are toxic and therefore are not recommended.

CLEAN-UP OF BIOHAZARDOUS SPILLS

A. BIOHAZARDOUS SPILL INSIDE A BIOLOGICAL SAFETY CABINET

Chemical decontamination procedures should be initiated at once while the cabinet continues to operate to prevent escape of contaminants from the cabinet. Be careful with paper towels which can be sucked into the blower fan or HEPA filters.

1. Spray or wipe walls, work surfaces, and equipment with an appropriate disinfectant detergent, (e.g., 1:10 dilution of household bleach and 0.7% soap). A disinfectant detergent has the advantage of detergent activity, which is important because extraneous organic substances frequently interfere with the reaction between the microorganism and the active agent of the disinfectant. The operator should wear gloves during this procedure.

2. Flood the top work surface tray, and, if a Class II cabinet, the drain pan below the work surface, with a disinfectant and allow to stand 20 minutes.

3. Remove excess disinfectant from the tray by wiping with a sponge or cloth soaked in a disinfectant. For Class II cabinets, drain the tray into the cabinet drain pan, lift out tray and removable exhaust grillework, and wipe off top and bottom (underside) surfaces with a sponge or cloth soaked in a disinfectant. Then replace the grillwork and drain disinfectant from the drain pan into an appropriate container and autoclave according to standard procedures. Gloves, cloth or sponge should be autoclaved and discarded into the biohazard box.

B. BIOHAZARDOUS SPILL OUTSIDE A BIOLOGICAL SAFETY CABINET

1. LARGE SPILL

   a. Do not breathe, leave the room immediately, and close the door.

   b. Notify supervisor, OSEH, and warn others not to enter the contaminated area. Post the OSEH temporary warning sign.

   c. Remove and put your contaminated garments into a container for autoclaving and thoroughly wash your hands and face.

   d. Wait 30 minutes to allow dissipation of spill-created aerosols by the room ventilation air changes.

e. Put on a long-sleeve gown with tight fitting cuffs, mask, and rubber gloves before reentering the room.

f. Pour an appropriate disinfectant solution (1:10 dilution of household bleach) around the spill and allow it to flow into the spill. Paper towels soaked with the disinfectant may be used to cover the area. To minimize aerosolization, avoid pouring the disinfectant solution directly onto the spill.

g. Let stand 20 minutes to allow an adequate contact time.

h. Using an autoclavable dust pan and squeegee, and forceps for sharp materials, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a autoclave bag lined deep autoclave pan. Cover the pan with a suitable cover and autoclave according to standard directions.

i. The dust pan, squeegee, and forceps should be placed in an autoclave bag and autoclaved according to standard directions. Contact of reusable items with non-autoclavable plastic bags should be avoided -- separation of the plastic after autoclaving can be very difficult.

j. Wash and mop adjacent area and spill area with appropriate disinfectant - detergent solution.

k. Remove and discard protective clothing. Shower with a germicidal soap.

2. SMALL SPILL

a. Cover spill with paper towels.

b. Flood spill with appropriate disinfectant using care not to cause spatter. Add disinfectant slowly to outer margin of spill and allow it to flow in.

c. Allow disinfectant to act for 20 to 30 minutes before cleaning up with more paper towels.

d. Discard materials (paper towels, gloves, and other wastes from clean-up into an autoclave bag and autoclave.

C. RADIOACTIVE BIOHAZARDOUS SPILL OUTSIDE A BIOLOGICAL SAFETY CABINET

(Refer to The Johns Hopkins Medical Institutions Radiation Safety Manual)

In the event that a biohazardous spill also involves a radiation hazard, the clean-up procedure may have to be modified, depending on an evaluation of the risk assessment of relative biological and radiological hazard.

Laboratories handling radioactive substances must contact the Radiation Safety Office for consultation.

The following procedure indicates suggested variations from the biohazard spill procedure (above) that should be considered when a radioactive biohazard spill occurs outside a biological safety cabinet.

1. Do not breathe, leave the room immediately and close the door.

2. Notify supervisor, OSEH, and warn others not to enter the contaminated area and to post the temporary warning sign.

3. Remove and put contaminated garments into a container for autoclaving and thoroughly wash hands and face.

4. Wait thirty minutes to allow dissipation of spill-created aerosols by the room ventilation air changes.

   Before clean-up procedures begin, the Radiation Safety Office should survey the spill for external radiation hazard to determine the relative degree of risk.

5. Put on a long-sleeve gown with tight fitting cuffs, mask, and rubber gloves before reentering the room.

6. Pour an appropriate disinfectant solution (1:10 dilution of household bleach) around the spill and allow it to flow into the spill. Paper towels soaked with the disinfectant may be used to cover the area. To minimize aerosolization, avoid pouring the disinfectant solution directly onto the spill.

7. Let stand 20 minutes to allow adequate disinfectant contact time.

8. In most cases, the spill will involve ^{14}C or ^{3}H, which present no external hazard. However, if more energetic beta or gamma emitters are involved, care must be taken to prevent hand and body radiation exposure. The Radiation Safety Office must make this determination before the clean-up is begun.

   a. If the Radiation Safety Office approves, the biohazard-handling procedure may begin: Using an autoclavable dust pan and squeegee, and forceps for sharp materials, transfer all contaminated materials (paper towels, glass, liquid, gloves, etc.) into a autoclave bag lined deep pan. Cover the pan with a suitable cover and autoclave according to standard directions.

   b. If the Radiation Safety Office does not approve; but determines that radioactive vapors may be released and thereby contaminate the autoclave, the material must not be autoclaved. In that case, add sufficient disinfectant solution to immerse the contents of the waste container. The cover should be sealed with waterproof tape, and the container stored and handled for disposal as radioactive waste. Radioactive warning symbols should be affixed to the waste container. As a general rule, autoclaving should be avoided.

9. If autoclaving has been approved, the dust pan, squeegee, and forceps should be placed in an autoclave bag and autoclaved according to standard directions. Contact of reusable items with plastic bags should be avoided -- separation of the plastic after autoclaving can be very difficult.

10. A final radioactive survey should be made of the spill area, dust pan, squeegee, and forceps with an appropriate radiation measurement technique.

SHIPMENT OF BIOLOGICAL MATERIALS

INTRODUCTION

The labeling requirements for biologicals, infectious substances, etiologic agents, etc. are currently in a state of flux. OSEH recommends that investigators employ an overnight delivery service that does business overseas, to ensure that labeling and packaging are in compliance with international standards. The Biosafety Officer can provide updated information on these requirements.

Because of potential hazards associated with the shipment of biohazardous materials, state and federal regulations control shipping practices. The regulations for shipment of etiologic agents and recombinant DNA materials state that:

"No person may knowingly transport or cause to be transported in interstate traffic, directly or indirectly, any material, including but not limited to, diagnostic specimens and biological products, which such person reasonably believes may contain an etiologic agent, unless such material is packaged to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation."

A. Shipment of biohazardous materials, within the U.S.A. and through importation and exportation, are subject to a variety of regulations involving five different regulatory agencies:

U.S. Postal Service [39 CFR, part III]
U.S. Public Health Service, [42 CFR, part 72]
U.S. Department of Transportation, [49 CFR, parts 171-179]
U.S. Department of Agriculture, [9 CFR, subchapters D & E]

B. Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the governmental agencies listed above.

C. For purposes of the NIH Recombinant DNA Guidelines (Federal Register, Vol. 55, No. 41, Thursday, March 1, 1990, pages 7447-7448):

1. Host organisms or viruses will be shipped as etiologic agents regardless of whether or not they contain recombinant DNA if they are regulated as human pathogens by the U.S. Public Health Service [42 CFR, part 72] or as animal pathogens or plant pests under the Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture [Titles 9 and 7 CFR, respectively].

2. Additionally, host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA.

D. DEFINITIONS (42 CFR Part 72 - Interstate Shipment of Etiologic Agents, 1980)

1. BIOMEDICAL MATERIALS
   Materials that are known to contain, or could contain, etiologic agents.

2. DIAGNOSTIC SPECIMENS
   Any human or animal material including, but not limited to excreta, secreta, blood and its components, tissue, and tissue fluids "which (the shipper) reasonably believes may contain an etiologic agent" [and may pose a danger to others, if they were exposed to it because of a transportation accident or mishap in handling], even if it is being shipped for purposes of diagnosis, must be packaged according to 42 CFR Part 72.3. Patient specimens that would be expected to contain an etiologic agent should be shipped according to 42 CFR Part 72.3 (Etiologic Agent). Materials which the shipper reasonably believes does not contain an etiologic agent should ship according to 42 CFR Part 72.2.

3. BIOLOGICAL PRODUCT
   A product prepared and manufactured in accordance with provisions of 9CFR Parts 102-104, 21CFR Parts 312 & 600-680, and 42 CFR 72.2.

4. ETIOLOGIC AGENTS [INFECTIOUS SUBSTANCES]
   A viable microorganism or its toxin that causes or may cause, human or animal disease. A culture or suspension including purified or partially purified spores or toxins that are themselves etiologic agents. Packaged according to 42 CFR Part 72.3.

E. PACKAGING OF DIAGNOSTIC SPECIMENS AND BIOLOGICAL PRODUCTS
   (42 CFR Part 72.2). Must withstand leakage of contents, shocks, pressure changes, etc.

F. PACKAGING OF ETIOLOGIC AGENTS [INFECTIOUS SUBSTANCES]
   (42 CFR Part 72.3) -DANGEROUS GOODS

1. Primary container is securely closed and watertight tube, vial, ampule, etc.

2. Primary container placed in a durable, watertight secondary container.

3. Several primary containers in one secondary container if total contents of primary containers don’t exceed 50 ml.

4. Absorbent material around and between primary and secondary containers. Should be nonparticulate and absorb entire contents of primary container(s).

5. Outer shipping container for secondary containers is constructed of fiberboard, of cardboard, wood, etc., not bags, envelopes, etc.

6. Single primary containers shall not contain more than 1,000 ml of material.

7. Two or more primary containers can be put into one secondary container, if total volume doesn't exceed 1,000 ml.

8. Maximum amount of etiologic agent in one outer shipping container may not exceed 4,000 ml.

9. Dry ice must be placed between secondary container(s) and the outer shipping container.

10. Etiologic agent "Infectious Materials" label must be placed on outer shipping container.

G. SPECIAL HANDLING

1. Some highly infectious materials must be shipped by registered mail with notification of receipt to the sender immediately upon delivery.

2. When the notice of receipt is not received within 5 days following anticipated delivery the sender must notify CDC.

H. FOREIGN QUARANTINE (42 CFR PART 71.54 and 72.3)

1. The CDC regulates importation of all etiologic agents and hosts and vectors of human disease. Failure to have an appropriate import permit may result in confiscation of a shipment at a port of entry.

2. Call CDC at (404) 639-3883 to get a permit application (Form 0.753).

I. ORGANISMS & VECTORS - USDA IMPORTS (9 CFR PART 122.2)

1. The USDA regulates importation of all animal-derived materials and biological materials that have been in contact with materials of animal origin.

2. This includes DNA/RNA extracts, recombinant proteins, microorganisms, and cell culture-derived materials such as viruses, monoclonal antibodies and ascites fluid that have been exposed to animal serum.

3. There are only four USDA-approved sources of bovine serum;

Australia	Canada
New Zealand	United States

4. Any cell line or virus prepared using serum from another source must be passaged at least five times in an approved serum before it may be imported into the U.S.

5. Monoclonal antibodies and ascites fluid derived from cultures grown in non-approved serum may be approved for importation if they have been dialyzed against a buffer of pH 5.5 or lower for a minimum of 30 minutes, or purified by affinity column chromatography.

6. Recombinant expression systems cannot be imported if they contain any animal-derived product such as albumin.

7. All shipments of biological materials must be accompanied by a declaration from the producer confirming that the appropriate criteria have been met.

8. Call USDA at (301) 436-8226 to get a permit application (VS Form 16-3). For cell cultures or their products another application (VS Form 16-7) is needed.

J. For further information on shipping etiologic agents, please contact:

1. Office of Safety and Environmental Health, Biosafety Officer;

2. Centers for Disease Control, ATTN: Biosafety Office, 1600 Clifton Road, Atlanta GA 30333, (404) 639-3883;

3. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington DC 20590, (202) 366-4545; or

4. Department of Agriculture, ATTN: Animal & Plant Health Inspection Service, 6505 Belcrest Road, Hyattsville MD 20782, (301) 436-7885 for Animal Pathogens, (301) 436-7612 for Plant Pests.

INTRODUCTION

These guidelines were published in the *Federal Register* on July 5, 1994. Later revisions are available from the Biosafety Officer.

The guidelines are included in the Biosafety Manual to:

Assist those individuals registering recombinant DNA projects,

Provide detailed descriptions of biosafety levels, and

Include guidelines on the classification of etiologic agents.

For additional details on biosafety levels and classification of etiologic agents, refer to the CDC NIH *Biosafety in Microbiological and Biomedical Laboratories*, 3rd Edition. This is available from the Biosafety Officer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) June 1994. These NIH Guidelines supersede all earlier versions and shall be in effect until further notice.

TABLE OF CONTENTS

Section I. Scope of the NIH Guidelines
Section I-A. Purpose
Section I-B. Definition of Recombinant DNA Molecules
Section I-C. General Applicability
Section I-D. General Definitions
Section II. Containment
Section III. Experiments Covered by the NIH Guidelines
Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Approval Before Initiation
Section III-B. Experiments that Require NIH/ORDA and Institutional Biosafety Committee Approval Before Initiation
Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms Per Kilgram Body Weight
Section III-B-2. Accelerated Review of Human Gene Transfer Experiments
Section III-B-3. Minor Modifications to Human Gene Transfer Experiments
Section III-C. Experiments that Require Institutional Biosafety Committee Approval Before Initiation
Section III-C-1. Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents as Host-Vector Systems
Section III-C-2. Experiments in which DNA from Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems.
Section III-C-3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems
Section III-C-4. Experiments Involving Whole Animals
Section III-C-5. Experiments Involving Whole Plants

Section III-C-6. Experiments Involving More than 10 Liters of Culture
Section III-C-7. Human Gene Transfer Experiments Not Covered by Section III-A-2, III-B-2, III-B-3, and Not Considered Exempt under Section V-U
Section III-D. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation
Section III-D-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus
Section III-D-2. Experiments Involving Whole Plants
Section III-E. Exempt Experiments
Section IV. Roles and Responsibilities
Section IV-A. Policy
Section IV-B. Responsibilities of the Institution
Section IV-B-1. General Information
Section IV-B-2. Institutional Biosafety Committee (IBC)
Section IV-B-3. Biological Safety Officer (BSO)
Section IV-B-4. Principal Investigator (PI)
Section IV-C. Responsibilities of the National Institutes of Health (NIH)
Section IV-C-1. NIH Director
Section IV-C-1-a. General Responsibilities
Section IV-C-1-b. Specific Responsibilities
Section IV-C-1-b-(1) Major Actions
Section IV-C-1-b-(2) Minor Actions
Section IV-C-2. Recombinant DNA Advisory Committee (RAC)
Section IV-C-3. Office of Recombinant DNA Activities (ORDA)
Section IV-C-4. Other NIH Components
Section IV-D. Compliance with the NIH Guidelines
Section IV-E. Voluntary Compliance
Section V. Footnotes and References of Sections I-IV
Appendix A. Exemptions under Section III-E-5--Sublists of Natural Exchangers
Appendix B. Classification of Etiologic Agents and Oncogenic Viruses on the Basis of Hazard
Appendix B-I. Class 1 Agents
Appendix B-II. Class 2 Agents
Appendix B-III. Class 3 Agents
Appendix B-IV. Class 4 Agents
Appendix B-V. Class 5 Agents
Appendix B-VI. Footnotes and References of Appendix B
Appendix C. Exemptions under Section III-E-6
Appendix C-I. Recombinant DNA in Tissue Culture
Appendix C-II. Escherichia coli K-12 Host-Vector Systems
Appendix C-III. Saccharomyces Host-Vector Systems
Appendix C-IV. Bacillus subtilis or Bacillus licheniformis Host-Vector Systems
Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms
Appendix C-VI. Footnotes and References of Appendix C
Appendix D. Major Actions Taken under the NIH Guidelines
Appendix E. Certified Host-Vector Systems
Appendix E-I. Bacillus subtilis
Appendix E-II. Saccharomyces cerevisiae
Appendix E-III. Escherichia coli
Appendix E-IV. Neurospora crassa
Appendix E-V. Streptomyces
Appendix E-VI. Pseudomonas putida
Appendix F. Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates
Appendix F-I. General Information

Appendix F-II. Cloning of Toxin Molecule Genes in Escherichia coli K-12
Appendix F-III. Cloning of Toxic Molecule Genes in Organisms other than Escherichia coli K-12
Appendix F-IV. Specific Approvals
Appendix G. Physical Containment
Appendix G-I. Standard Practices and Training
Appendix G-II. Physical Containment Levels
Appendix G-II-A. Biosafety Level 1 (BL1)
Appendix G-II-B. Biosafety Level 2 (BL2)
Appendix G-II-C. Biosafety Level 3 (BL3)
Appendix G-II-D. Biosafety Level 4 (BL4)
Appendix G-III. Footnotes and References of Appendix G.
Appendix H. Shipment
Appendix I. Biological Containment
Appendix I-I. Levels of Biological Containment
Appendix I-I-A. Host-Vector 1 Systems
Appendix I-I-B. Host-Vector 2 Systems
Appendix I-II. Certification of Host-Vector Systems
Appendix I-III. Footnotes and References of Appendix I
Appendix J. Biotechnology Research Subcommittee
Appendix K. Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules
Appendix K-I. Selection of Physical Containment Levels
Appendix K-II. Good Large Scale Practices (GLSP)
Appendix K-III. Biosafety Level 1 (BL1) - Large Scale
Appendix K-IV. Biosafety Level 2 (BL2) - Large Scale
Appendix K-V. Biosafety Level 3 (BL3) - Large Scale
Appendix K-VI. Footnotes of Appendix K
Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K
Appendix L. Release into the Environment of Certain Plants
Appendix M. Points to Consider in the Design and Submission of Protocols for the Transfer of Recombinant DNA Molecules into the Genome of One or More Human Subjects
Appendix M-I. Description of Proposal
Appendix M-I-A. Objectives and Rationale of the Proposed Research
Appendix M-I-B. Research Design, Anticipated Risks and Benefits
Appendix M-I-C. Selection of the Patients
Appendix M-I-D. Informed Consent
Appendix M-I-E. Privacy and Confidentiality
Appendix M-II. Special Issues
Appendix M-III. Guidelines for the Submission of Human Gene Transfer Protocols
Appendix M-III-A. Principal Investigator-Submitted Material
Appendix M-III-B. Time Frame for Submissions
Appendix M-III-C. Oral Responses to the RAC
Appendix M-III-D. Primary Reviewers' Responses
Appendix M-IV. Reporting Requirements
Appendix M-V. Procedures to the Followed for Accelerated Review of Human Gene Transfer Experiments by NIH/ORDA under Section III-B-2
Appendix M-VI. Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments by the NIH Director Under Section III-A-2
Appendix M-VII. Footnotes of Appendix M
Appendix P. Physical and Biological Containment for Recombinant DNA Research Involving Plants
Appendix P-I. General Plant Biosafety Levels
Appendix P-II. Physical Containment Levels
Appendix P-II-A. Biosafety Level 1 - Plants (BL1-P)
Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P)

Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P)
Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P)
Appendix P-III. Biological Containment Practices
Appendix P-III-A. Biological Containment Practices (Plants)
Appendix P-III-B. Biological Containment Practices (Microorganisms)
Appendix P-III-C. Biological Containment Practices (Macroorganisms)
Appendix Q. Physical and Biological Containment for Recombinant DNA Research Involving Animals
Appendix Q-I. General Considerations
Appendix Q-I-A. Containment Levels
Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N)
Appendix Q-II. Physical and Biological Containment Levels
Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N)
Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N)
Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N)
Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N)
Appendix Q-III. Footnotes and References for Appendix Q

SECTION I. SCOPE OF THE NIH GUIDELINES

Section I-A. Purpose

The purpose of the NIH Guidelines is to specify practices for constructing and handling: (i) recombinant deoxyribonucleic acid (DNA) molecules, and (ii) organisms and viruses containing recombinant DNA molecules.

Section I-A-1. Any recombinant DNA experiment, which according to the NIH Guidelines requires approval by the NIH, must be submitted to the NIH or to another Federal agency that has jurisdiction for review and approval. Once approval, or other applicable clearances, has been obtained from a Federal agency other than the NIH (whether the experiment is referred to that agency by the NIH or sent directly there by the submitter), the experiment may proceed without the necessity for NIH review or approval (see exceptions in Sections I-A-2 and I-A-3).

Section I-A-2. Certain experiments that involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V-U) shall be considered Major Actions (see Section IV-C-1-b-(1)), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary, to: (i) represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review (see Section III-A-2).

Section I-A-3. Experiments involving the transfer of recombinant DNA to one or more human subjects that are not considered under Section III-A-2 may qualify for Accelerated Review (see Section III-B-2 and Appendix M-V) and will be considered as Minor Actions (see Section IV-C-1-b-(2)-(a)). Actions that qualify for Accelerated Review will be reviewed and approved by NIH/ORDA in consultation with the RAC Chair and/or one or more RAC members, as necessary.

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V-U) may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III-C-7).

Section I-B. Definition of Recombinant DNA Molecules

In the context of the NIH Guidelines, recombinant DNA molecules are defined as either: (i) molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above.

Synthetic DNA segments which are likely to yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent) are considered as equivalent to their natural DNA counterpart. If the synthetic DNA segment is not expressed in vivo as a biologically active polynucleotide or polypeptide product, it is exempt from the NIH Guidelines.

Genomic DNA of plants and bacteria that have acquired a transposable element, even if the latter was donated from a recombinant vector no longer present, are not subject to the NIH Guidelines unless the transposon itself contains recombinant DNA.

Section I-C. General Applicability

Section I-C-1. The NIH Guidelines are applicable to:

Section I-C-1-a. All recombinant DNA research within the United States (U.S.) or its territories that is conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH, including research performed directly by the NIH. An individual who receives support for research involving recombinant DNA must be associated with or sponsored by an institution that assumes the responsibilities assigned in the NIH Guidelines.

Section I-C-1-b. All recombinant DNA research performed abroad: Specifically:

Section I-C-1-b-(1). Research supported by NIH funds.

Section I-C-1-b-(2). If they involve testing in humans of materials containing recombinant DNA developed with NIH funds and if the institution that developed those materials sponsors or participates in those projects. Participation includes research collaboration or contractual agreements, not mere provision of research materials.

Section I-C-1-b-(3). If the host country has established rules for the conduct of recombinant DNA research, then the research must be in compliance with those rules. If the host country does not have such rules, the proposed research must be reviewed and approved by an NIH-approved Institutional Biosafety Committee or equivalent review body and accepted in writing by an appropriate national governmental authority of the host country. The safety practices that are employed abroad must be reasonably consistent with the NIH Guidelines.

Section I-D. General Definitions

The following terms, which are used throughout the NIH Guidelines, are defined as follows:

Section I-D-1. An "institution" is any public or private entity (including Federal, state, and local government agencies).

Section I-D-2. An "Institutional Biosafety Committee" is a committee that: (i) meets the requirements for membership specified in Section IV-B-2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Section IV-B-2.

Section I-D-3. The "Office of Recombinant DNA Activities (ORDA)" is the office within the NIH that is responsible for: (i) reviewing and coordinating all activities relating to the NIH Guidelines, and (ii) performing other duties as defined in Section IV-C-3.

Section I-D-4. The "Recombinant DNA Advisory Committee" is the public advisory committee that advises the Department of Health and Human Services (DHHS) Secretary, the DHHS Assistant Secretary for Health, and the NIH Director concerning recombinant DNA research. The RAC shall be constituted as specified in Section IV-C-2.

Section I-D-5. The "NIH Director" is the Director of the National Institutes of Health, or any other officer or employee of NIH to whom authority has been delegated.

Section I-D-6. "Deliberate release" is defined as a planned introduction of recombinant DNA-containing microorganisms, plants, or animals into the environment.

SECTION II. CONTAINMENT

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information already exists about the design of physical containment facilities and selection of laboratory procedures applicable to organisms carrying recombinant DNA (see Section V-A). The existing programs rely upon mechanisms that can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories; and (ii) special procedures, equipment, and laboratory installations that provide physical barriers that are applied in varying degrees according to the estimated biohazard. Four biosafety levels are described in Appendix G. These biosafety levels consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and are based on the potential hazards imposed by the agents used and for the laboratory function and activity. Biosafety Level 4 provides the most stringent containment conditions, Biosafety Level 1 the least stringent.

Experiments involving recombinant DNA lend themselves to a third containment mechanism, namely, the application of highly specific biological barriers. Natural barriers exist that limit either: (i) the infectivity of a vector or vehicle (plasmid or virus) for specific hosts, or (ii) its dissemination and survival in the environment. Vectors, which provide the means for recombinant DNA and/or host cell replication, can be genetically designed to decrease, by many orders of magnitude, the probability of dissemination of recombinant DNA outside the laboratory (see Appendix I).

Since these three means of containment are complementary, different levels of containment can be established that apply various combinations of the physical and biological barriers along with a constant use of standard practices. Categories of containment are considered separately in order that such combinations can be conveniently expressed in the NIH Guidelines.

Physical containment conditions within laboratories, described in Appendix G, may not always be appropriate for all organisms because of their physical size, the number of organisms needed for an experiment, or the particular growth requirements of the organism. Likewise, biological containment for microorganisms described in Appendix I may not be appropriate for all organisms, particularly higher eukaryotic organisms. However, significant information exists about the design of research facilities and experimental procedures that are applicable to organisms containing recombinant DNA that is either integrated into the genome or into microorganisms associated with the higher organism as a symbiont, pathogen, or other relationship. This information describes facilities for physical containment of organisms used in non-traditional laboratory settings and special practices for limiting or excluding the unwanted establishment, transfer of genetic information, and dissemination of organisms beyond the intended location, based on both physical and biological containment principles. Research conducted in accordance with these conditions effectively confines the organism.

For research involving plants, four biosafety levels (BL1-P through BL4-P) are described in Appendix P. BL1-P is designed to provide a moderate level of containment for experiments for which there is convincing biological evidence that precludes the possibility of survival, transfer, or dissemination of recombinant DNA into the environment, or in which there is no recognizable and predictable risk to the environment in the event of accidental release. BL2-P is designed to provide a greater level of containment for experiments involving plants and certain associated organisms in which there is a recognized possibility of survival, transmission, or dissemination of recombinant DNA containing organisms, but the consequence of such an inadvertent release has a predictably minimal biological impact. BL3-P and BL4-P describe additional containment conditions for research with plants and certain pathogens and other organisms that require special containment because of their recognized potential for significant detrimental impact on managed or natural ecosystems. BL1-P relies upon accepted scientific practices for conducting research in most ordinary greenhouse or growth chamber facilities and incorporates accepted procedures for good pest control and cultural practices. BL1-P facilities and procedures provide a modified and protected environment for the propagation of plants and microorganisms associated with the plants and a degree of containment that adequately controls the potential for release of biologically viable plants, plant parts, and microorganisms associated with them. BL2-P and BL3-P rely upon accepted scientific practices for conducting research in greenhouses with organisms infecting or infesting plants in a manner that minimizes or prevents inadvertent contamination of plants within or surrounding the greenhouse. BL4-P describes facilities and practices known to provide containment of certain exotic plant pathogens.

For research involving animals, which are of a size or have growth requirements that preclude the use of conventional primary containment systems used for small laboratory animals, four biosafety levels (BL1-N through BL4-N) are described in Appendix Q. BL1-N describes containment for animals that have been modified by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms and is designed to eliminate the possibility of sexual transmission of the modified genome or transmission of recombinant DNA-derived viruses known to be transmitted from animal parent to offspring only by sexual reproduction. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals. BL2-N describes containment which is used for transgenic animals associated with recombinant DNA-derived organisms and is designed to eliminate the possibility of vertical or horizontal transmission. Procedures, practices, and facilities follow classical methods of avoiding genetic exchange between animals or controlling arthropod transmission. BL3-N and BL4-N describe higher levels of containment for research with certain transgenic animals involving agents which pose recognized hazard.

In constructing the NIH Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. These definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be conducted under different conditions than indicated here without affecting risk.
Individual investigators and Institutional Biosafety Committees are urged to devise simple and more effective containment procedures and to submit recommended changes in the NIH Guidelines to permit the use of these procedures.

SECTION III. EXPERIMENTS COVERED BY THE NIH GUIDELINES

This section describes five categories of experiments involving recombinant DNA: (i) those that require RAC review and NIH and Institutional Biosafety Committee approval before initiation (see Section III-A), (ii) those that require NIH/ORDA and Institutional Biosafety Committee approval before initiation (see Section III-B); (iii) those that require Institutional Biosafety Committee approval before initiation (see Section III-C), (iv) those that require Institutional Biosafety Committee notification simultaneous with initiation (see Section III-D), and (v) those that are exempt from the NIH Guidelines (see Section III-E).

Note: If an experiment falls into either Section III-A or Section III-B and one of the other categories, the rules pertaining to Section III-A or Section III-B shall be followed. If an experiment falls into Section III-E and into either Sections III-C or III-D categories as well, the experiment is considered exempt from the NIH Guidelines.

Any change in containment level, which is different from those specified in the NIH Guidelines, may not be initiated without the express approval of NIH/ORDA (see Minor Actions, Section IV-C-1-b-(2) and its subsections).

Section III-A. Experiments that Require Institutional Biosafety Committee Approval, RAC Review, and NIH Approval Before Initiation

Experiments in this category are considered Major Actions (see Section IV-C-1-b-(1)) and cannot be initiated without submission of relevant information on the proposed experiment to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838, the publication of the proposal in the Federal Register for 15 days of comment, reviewed by the RAC, and specific approval by the NIH (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M-VI). The containment conditions for such experiments will be recommended by the RAC and set by the NIH at the time of approval. Such experiments require Institutional Biosafety Committee approval before initiation. Specific experiments already approved are included in Appendix D which may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section III-A-1. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire the trait naturally (see Section V-B), if such acquisition could compromise the use of the drug to control disease agents in humans, veterinary medicine, or agriculture.

Section III-A-2. Certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects (see Section V-U) shall be considered Major Actions (see Section IV-C-1-b-(1) and Appendix M-III), and shall require RAC review and NIH Director approval, if determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. The requirement for RAC review shall not be considered to preempt any other required review or approval of experiments with one or more human subjects. Relevant Institutional Biosafety Committee and Institutional Review Board reviews and approvals of the proposal should be completed before submission to NIH. Certain experiments involving deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects may qualify for the Accelerated Review process (see Section III-B-2). Certain categories of experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and that are not covered by Section V-U, may be considered exempt from RAC and/or NIH/ORDA review and/or NIH Director approval and only require registration with NIH/ORDA (see Section III-C-7).

Section III-B. Experiments That Require NIH/ORDA and Institutional Biosafety Committee Approval Before Initiation

Section III-B-1. Experiments Involving the Cloning of Toxin Molecules with LD50 of Less than 100 Nanograms per Kilogram Body Weight

Deliberate formation of recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of less than 100 nanograms per kilogram body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, and Shigella dysenteriae neurotoxin). Specific approval has been given for the cloning in Escherichia coli K-12 of DNA containing genes coding for the biosynthesis of toxic molecules which are lethal to vertebrates at 100 nanograms to 100 micrograms per kilogram body weight. Specific experiments already approved under this section may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section III-B-1-(a). Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH/ORDA. The containment conditions for such experiments will be determined by NIH/ORDA in consultation with ad hoc experts. Such experiments require Institutional Biosafety Committee approval before initiation (see Section IV-B-2-b-(1)).

Section III-B-2. Accelerated Review of Human Gene Transfer Experiments

As determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, certain categories of human gene transfer experiments may be considered as Minor Actions and qualify for Accelerated Review and approval (see Section IV-C-1-b-(2)-(a), Appendix M-III-A, and Appendix M-V). The RAC Chair will present a report of all NIH/ORDA approved human gene transfer protocols at the next regularly scheduled RAC meeting. If NIH/ORDA determines that an experiment does not qualify for the Accelerated Review process, the Principal Investigator must submit the proposal for full RAC review $\geq$ 8 weeks prior to the next scheduled RAC meeting (See Section III-A-2).

Section III-B-3. Minor Modifications to Human Gene Transfer Experiments

A minor modification in a human gene transfer protocol is a modification that does not significantly alter the basic design of the protocol and that does not increase risk to human subjects or the environment. After approval has been obtained by the relevant Institutional Biosafety Committee and Institutional Review Board, NIH/ORDA will consider the change in consultation with the RAC Chair and one or more RAC members, as necessary. Submit minor modifications to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. The RAC Chair will provide a report on any such approvals at the next regularly scheduled RAC meeting.

Section III-C. Experiments that Require Institutional Biosafety Committee Approval Before Initiation

Prior to the initiation of an experiment that falls into this category, the Principal Investigator must submit a registration document to the Institutional Biosafety Committee which contains the following information: (i) the source(s) of DNA; (ii) the nature of the inserted DNA sequences; (iii) the host(s) and vector(s) to be used; (iv) if an attempt will be made to obtain expression of a foreign gene, and if so, indicate the protein that will be produced; and (v) the containment conditions that will be implemented as specified in the NIH Guidelines. For experiments in this category, the registration document shall be dated, signed by the Principal Investigator, and filed with the Institutional Biosafety Committee. The Institutional Biosafety Committee shall review and approve all experiments in this category prior to their initiation. Requests to decrease the level of containment specified for experiments in this category will be considered by NIH (see Section IV-C-1-b-(2)-(c)).

Section III-C-1. Experiments Using Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (see Section V-A) as Host-Vector Systems

Section III-C-1-a. Experiments involving the introduction of recombinant DNA into Class 2 agents shall be conducted at Biosafety Level (BL) 2 containment. Experiments with such agents shall be conducted with whole animals at BL2 or BL2-N (Animals) containment.

Section III-C-1-b. Experiments involving the introduction of recombinant DNA into Class 3 agents shall be conducted at BL3 containment. Experiments with such agents shall be conducted with whole animals at BL3 or BL3-N containment.

Section III-C-1-c. Experiments involving the introduction of recombinant DNA into Class 4 agents shall be conducted at BL4 containment. Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-C-1-d. Containment conditions for experiments involving the introduction of recombinant DNA into Class 5 agents shall be set on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V-R and V-T). Experiments with such agents shall be conducted with whole animals at BL4 or BL4-N containment.

Section III-C-2. Experiments in Which DNA From Human or Animal Pathogens (Class 2, Class 3, Class 4, or Class 5 Agents (see Section V-A) is Cloned into Nonpathogenic Prokaryotic or Lower Eukaryotic Host-Vector Systems

Section III-C-2-a. Experiments in which DNA from Class 2 or Class 3 agents (see Section V-A) is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment. Experiments in which DNA from Class 4 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes may be performed under BL2 containment after demonstration that only a totally and irreversibly defective fraction of the agent's genome is present in a given recombinant. In the absence of such a demonstration, BL4 containment shall be used. The Institutional Biosafety Committee may approve the specific lowering of containment for particular experiments to BL1. Many experiments in this category are exempt from the NIH Guidelines (see Section III-E). Experiments involving the formation of recombinant DNA for certain genes coding for molecules toxic for vertebrates require NIH/ORDA approval (see Section III-B-1) or shall be conducted under NIH specified conditions as described in Appendix F.

Section III-C-2-b. Containment conditions for experiments in which DNA from Class 5 agents is transferred into nonpathogenic prokaryotes or lower eukaryotes shall be determined by NIH/ORDA following a case-by-case review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V-R and V-T).

Section III-C-3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions that permit a productive infection. In such cases, serious consideration should be given to increasing physical containment by at least one level.

Note: Recombinant DNA or RNA molecules derived therefrom, which contain less than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-Q) being considered identical (see Section V-S), are considered defective and may be used in the absence of helper under the conditions specified in Section III-D-1.

Section III-C-3-a. Experiments involving the use of infectious or defective Class 2 animal viruses (see Section V-A, Appendix B-II, and Appendix B-II-E) in the presence of helper virus may be conducted at BL2.

Section III-C-3-b. Experiments involving the use of infectious or defective Class 3 animal viruses (see Section V-A and Appendix B-III-D) in the presence of helper virus may be conducted at BL3.

Section III-C-3-c. Experiments involving the use of infectious or defective Class 4 animal viruses (see Section V-A and Appendix B-IV-D) in the presence of helper virus may be conducted at BL4.

Section III-C-3-d. Experiments involving the use of infectious or defective Class 5 viruses (see Section V-A and Appendix B-V) in the presence of helper virus shall be determined on a case-by-case basis following NIH/ORDA review. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Sections V-R and V-T).

Section III-C-3-e. Experiments involving the use of infectious or defective animal or plant viruses in the presence of helper virus are not covered in Sections III-C-3-a through III-C-3-d and may be conducted at BL1.

Section III-C-4. Experiments Involving Whole Animals

This section covers experiments involving whole animals in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. For the latter, other than viruses which are only vertically transmitted, the experiments may not be conducted at BL1-N containment. A minimum containment of BL2 or BL2-N is required.

CAUTION - Special care should be used in the evaluation of containment conditions for some experiments with transgenic animals. For example, such experiments might lead to the creation of novel mechanisms or increased transmission of a recombinant pathogen or production of undesirable traits in the host animal. In such cases, serious consideration should be given to increasing the containment conditions.

Section III-C-4-a. Recombinant DNA, or DNA or RNA molecules derived therefrom, from any source except for greater than two-thirds of eukaryotic viral genome may be transferred to any non-human vertebrate or any invertebrate organism and propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study (see Section V-B). Animals that contain sequences from viral vectors, which do not lead to transmissible infection either directly or indirectly as a result of complementation or recombination in animals, may be propagated under conditions of physical containment comparable to BL1 or BL1-N and appropriate to the organism under study. Experiments involving the introduction of other sequences from eukaryotic viral genomes into animals are covered under SectionIII-C-4-b. For experiments involving recombinant DNA-modified Class 2, 3, 4, or 5 organisms, see Section V-A. It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A U.S. Department of Agriculture permit is required for work with Class 5 agents (see Section V-R and V-T).

Section III-C-4-b. For experiments involving recombinant DNA, or DNA or RNA derived therefrom, involving whole animals, including transgenic animals, and not covered by Sections III-C-1 or III-C-4-a, the appropriate containment shall be determined by the Institutional Biosafety Committee.

Section III-C-5. Experiments Involving Whole Plants

Experiments to genetically engineer plants by recombinant DNA methods, to use such plants for other experimental purposes (e.g., response to stress), to propagate such plants, or to use plants together with microorganisms or insects containing recombinant DNA, may be conducted under the containment conditions described in Sections III-C-5-a through III-C-5-e. If experiments involving whole plants are not described in Section III-C-5 and do not fall under Sections III-A, III-B, or III-E, they are included in Section III-D.

NOTE - For recombinant DNA experiments falling under Sections III-C-5-a through III-C-5-d, physical containment requirements may be reduced to the next lower level by appropriate biological containment practices, such as conducting experiments on a virus with an obligate insect vector in the absence of that vector or using a genetically attenuated strain.

Section III-C-5-a. BL3-P (Plants) or BL2-P + biological containment is recommended for experiments involving most exotic (see Section V-W) infectious agents with recognized potential for serious detrimental impact on managed or natural ecosystems when recombinant DNA techniques are associated with whole plants.

Section III-C-5-b. BL3-P or BL2-P + biological containment is recommended for experiments involving plants containing cloned genomes of readily transmissible exotic (see Section V-W) infectious agents with recognized potential for serious detrimental effects on managed or natural ecosystems in which there exists the possibility of reconstituting the complete and functional genome of the infectious agent by genomic complementation in planta.

Section III-C-5-c. BL4-P containment is recommended for experiments with a small number of readily transmissible exotic (see Section V-W) infectious agents, such as the soybean rust fungus (Phakospora pachyrhizi) and maize streak or other viruses in the presence of their specific arthropod vectors, that have the potential of being serious pathogens of major U.S. crops.

Section III-C-5-d. BL3-P containment is recommended for experiments involving sequences encoding potent vertebrate toxins introduced into plants or associated organisms. Recombinant DNA containing genes for the biosynthesis of toxin molecules lethal for vertebrates at an LD50 of <100 nanograms per kilogram body weight fall under Section III-B-1 and require NIH/ORDA and Institutional Biosafety Committee approval before initiation.

Section III-C-5-e. BL3-P or BL2-P + biological containment is recommended for experiments with microbial pathogens of insects or small animals associated with plants if the recombinant DNA- modified organism has a recognized potential for serious detrimental impact on managed or natural ecosystems.

Section III-C-6. Experiments Involving More than 10 Liters of Culture

The appropriate containment will be decided by the Institutional Biosafety Committee. Where appropriate, Appendix K, Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules, shall be used. Appendix K describes containment conditions Good Large Scale Practice through BL3-Large Scale.

Section III-C-7. Human Gene Transfer Experiments Not Covered by Sections III-A-2, III-B-2, III-B-3, and Not Considered Exempt Under Section V-U

Certain experiments involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that are not covered by Sections III-A-2, III-B-2, III-B-3, and that are not considered exempt under Section V-U must be registered with NIH/ORDA. The relevant Institutional Biosafety Committee and Institutional Review Board must review and approve all experiments in this category prior to their initiation.

Section III-D. Experiments that Require Institutional Biosafety Committee Notice Simultaneous with Initiation

Experiments not included in Sections III-A, III-B, III-C, III-E, and their subsections are considered in Section III-D. All such experiments may be conducted at BL1 containment. For experiments in this category, a registration document (see Section III-C) shall be dated and signed by the investigator and filed with the local Institutional Biosafety Committee at the time the experiment is initiated. The Institutional Biosafety Committee reviews and approves all such proposals, but Institutional Biosafety Committee review and approval prior to initiation of the experiment is not required (see Section IV-A). For example, experiments in which all components derived from

non-pathogenic prokaryotes and non-pathogenic lower eukaryotes fall under Section III-D and may be conducted at BL1 containment.

Section III-D-1. Experiments Involving the Formation of Recombinant DNA Molecules Containing No More than Two-Thirds of the Genome of any Eukaryotic Virus

Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (see Section V-Q) being considered identical (see Section V-S)) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be demonstrated that the cells lack helper virus for the specific Families of defective viruses being used. If helper virus is present, procedures specified under Section III-C-3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment shall be less than two-thirds of a genome.

Section III-D-2. Experiments Involving Whole Plants

This section covers experiments involving recombinant DNA-modified whole plants, and/or experiments involving recombinant DNA-modified organisms associated with whole plants, except those that fall under Section III-A, III-B, III-C, or III-E. It should be emphasized that knowledge of the organisms and judgment based on accepted scientific practices should be used in all cases in selecting the appropriate level of containment. For example, if the genetic modification has the objective of increasing pathogenicity or converting a non-pathogenic organism into a pathogen, then a higher level of containment may be appropriate depending on the organism, its mode of dissemination, and its target organisms. By contrast, a lower level of containment may be appropriate for small animals associated with many types of recombinant DNA-modified plants.

Section III-D-2-a. BL1-P is recommended for all experiments with recombinant DNA-containing plants and plant-associated microorganisms not covered in Section III-D-2-b or other sections of the NIH Guidelines. Examples of such experiments are those involving recombinant DNA-modified plants that are not noxious weeds or that cannot interbreed with noxious weeds in the immediate geographic area, and experiments involving whole plants and recombinant DNA-modified non-exotic (see Section V-W) microorganisms that have no recognized potential for rapid and widespread dissemination or for serious detrimental impact on managed or natural ecosystems (e.g., Rhizobium spp. and Agrobacterium spp.).

Section III-D-2-b. BL2-P or BL1-P + biological containment is recommended for the following experiments:

Section III-D-2-b-(1). Plants modified by recombinant DNA that are noxious weeds or can interbreed with noxious weeds in the immediate geographic area.

Section III-D-2-b-(2). Plants in which the introduced DNA represents the complete genome of a non-exotic infectious agent (see Section V-W).

Section III-D-2-b-(3). Plants associated with recombinant DNA-modified non-exotic microorganisms that have a recognized potential for serious detrimental impact on managed or natural ecosystems (see Section V-W).

Section III-D-2-b-(4). Plants associated with recombinant DNA-modified exotic microorganisms that have no recognized potential for serious natural ecosystems (see Section V-W).

Section III-D-2-b-(5). Experiments with recombinant DNA-modified arthropods or small animals associated with plants, or with arthropods or small animals with recombinant DNA-modified microorganisms associated with them if the recombinant DNA-modified microorganisms have no recognized potential for serious detrimental impact on managed or natural ecosystems (see Section
V-W).

Section III-E. Exempt Experiments

The following recombinant DNA molecules are exempt from the NIH Guidelines and registration with the Institutional Biosafety Committee is not required:

Section III-E-1. Those that are not in organisms or viruses.

Section III-E-2. Those that consist entirely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

Section III-E-3. Those that consist entirely of DNA from a prokaryotic host including its indigenous plasmids or viruses when propagated only in that host (or a closely related strain of the same species), or when transferred to another host by well established physiological means.

Section III-E-4. Those that consist entirely of DNA from an eukaryotic host including its chloroplasts, mitochondria, or plasmids (but excluding viruses) when propagated only in that host (or a closely related strain of the same species).

Section III-E-5. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice of the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c)). See Appendices A-I through A-VI for a list of natural exchangers that are exempt from the NIH Guidelines.

Section III-E-6. Those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C for other classes of experiments which are exempt from the NIH Guidelines.

SECTION IV. ROLES AND RESPONSIBILITIES

Section IV-A. Policy

The safe conduct of experiments involving recombinant DNA depends on the individual conducting such activities. The NIH Guidelines cannot anticipate every possible situation. Motivation and good judgment are the key essentials to protection of health and the environment. The NIH Guidelines are intended to assist the institution, Institutional Biosafety Committee, Biological Safety Officer, and Principal Investigator in determining safeguards that should be implemented. The NIH Guidelines will never be complete or final since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the institution and those associated with it to adhere to the intent of the NIH Guidelines as well as to their specifics. Each institution (and the Institutional Biosafety Committee acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the NIH Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level. The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by the NIH as necessary.

Section IV-B. Responsibilities of the Institution

Section IV-B-1. General Information

Each institution conducting or sponsoring recombinant DNA research which is covered by the NIH Guidelines is responsible for ensuring that the research is conducted in full conformity with the provisions of the NIH Guidelines. In order to fulfill this responsibility, the institution shall:

Section IV-B-1-a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the NIH Guidelines. As part of its general responsibilities for implementing the NIH Guidelines, the institution may establish additional procedures, as deemed necessary, to govern the institution and its components in the discharge of its responsibilities under the NIH Guidelines. Such procedures may include: (i) statements formulated by the institution for the general implementation of the NIH Guidelines, and (ii) any additional precautionary steps the institution deems appropriate.

Section IV-B-1-b. Establish an Institutional Biosafety Committee that meets the requirements set forth in Section IV-B-2-a and carries out the functions detailed in Section IV-B-2-b.

Section IV-B-1-c. Appoint a Biological Safety Officer (who is also a member of the Institutional Biosafety Committee) if the institution: (i) conducts recombinant DNA research at Biosafety Level (BL) 3 or BL4, or (ii) engages in large scale (greater than 10 liters) research. The Biological Safety Officer carries out the duties specified in Section IV-B-3.

Section IV-B-1-d. Assist and ensure compliance with the NIH Guidelines by Principal Investigators conducting research at the institution as specified in Section IV-B-4.

Section IV-B-1-e. Ensure appropriate training for the Institutional Biosafety Committee Chair and members, Biological Safety Officer (when applicable), Principal Investigators, and laboratory staff regarding laboratory safety and implementation of the NIH Guidelines. The Institutional Biosafety Committee Chair is responsible for ensuring that Institutional Biosafety Committee members are appropriately trained. The Principal Investigator is responsible for ensuring that laboratory staff are appropriately trained. The institution is responsible for ensuring that the Principal Investigator has sufficient training; however, this responsibility may be delegated to the Institutional Biosafety Committee.

Section IV-B-1-f. Determine the necessity for health surveillance of personnel involved in connection with individual recombinant DNA projects; and if appropriate, conduct a health surveillance program for such projects. The institution shall establish and maintain a health surveillance program for personnel engaged in large scale research or production activities involving viable organisms containing recombinant DNA molecules which require BL3 containment at the laboratory scale. The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require BL3 or greater containment in the laboratory. The Laboratory Safety Monograph discusses various components of such a program (e.g., records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience). Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples cited in the Laboratory Safety Monograph include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness. Copies of the Laboratory Safety Monograph are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section IV-B-1-g. Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to NIH/ORDA within thirty days, unless the institution determines that a report has already been filed by the Principal Investigator or Institutional Biosafety Committee. Reports shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section IV-B-2. Institutional Biosafety Committee (IBC)

The institution shall establish an Institutional Biosafety Committee whose responsibilities need not be restricted to recombinant DNA. The Institutional Biosafety Committee shall meet the following requirements:

Section IV-B-2-a. Membership and Procedures

Section IV-B-2-a-(1). The Institutional Biosafety Committee must be comprised of no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research and to identify any potential risk to public health or the environment. At least two members shall not be affiliated with the institution (apart from their membership on the Institutional Biosafety Committee) and who represent the interest of the surrounding community with respect to health and protection of the environment (e.g., officials of state or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community). The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee. The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval. When the institution conducts recombinant DNA research at BL3 or BL4, a Biological Safety Officer is mandatory and shall be a member of the Institutional Biosafety Committee (see Section IV-B-3).

Section IV-B-2-a-(2). In order to ensure the competence necessary to review and approve recombinant DNA activities, it is recommended that the Institutional Biosafety Committee: (i) include persons with expertise in recombinant DNA technology, biological safety, and physical containment; (ii) include or have available as consultants persons knowledgeable in institutional commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment, and (iii) include at least one member representing the laboratory technical staff.

Section IV-B-2-a-(3). The institution shall file a report with NIH/ORDA which includes the names and biographical sketches of all Institutional Biosafety Committee members, including community members, in such form and at such times as required by NIH/ORDA.

Section IV-B-2-a-(4). No member of an Institutional Biosafety Committee may be involved (except to provide information requested by the Institutional Biosafety Committee) in the review or approval of a project in which he/she has been or expects to be engaged or has a direct financial interest.

Section IV-B-2-a-(5). The institution, that is ultimately responsible for the effectiveness of the Institutional Biosafety Committee, may establish procedures that the Institutional Biosafety Committee shall follow in its initial and continuing review and approval of applications, proposals, and activities.

Section IV-B-2-a-(6). When possible and consistent with protection of privacy and proprietary interests, the institution is encouraged to open its Institutional Biosafety Committee meetings to the public.

Section IV-B-2-a-(7). Upon request, the institution shall make available to the public all Institutional Biosafety Committee meeting minutes and any documents submitted to or received from funding agencies which the latter are required to make available to the public. If public comments are made on Institutional Biosafety Committee actions, the institution shall forward both the public comments and the Institutional Biosafety Committee's response to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Section IV-B-2-b. Functions

On behalf of the institution, the Institutional Biosafety Committee is responsible for:

Section IV-B-2-b-(1). Reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with the NIH Guidelines as specified in Section III and approving those research projects that are found to conform with the NIH Guidelines. This review shall include: (i) independent assessment of the containment levels required by the NIH Guidelines for the proposed research, and (ii) assessment of the facilities, procedures, practices, and training and expertise of personnel involved in recombinant DNA research.

Section IV-B-2-b-(2). Notifying the Principal Investigator of the results of the Institutional Biosafety Committee's review and approval.

Section IV-B-2-b-(3). Lowering containment levels for certain experiments as specified in Section III-C-2-a.

Section IV-B-2-b-(4). Setting containment levels as specified in Sections III-C-4-b and III-C-5.

Section IV-B-2-b-(5). Periodically reviewing recombinant DNA research conducted at the institution to ensure compliance with the NIH Guidelines.

Section IV-B-2-b-(6). Adopting emergency plans covering accidental spills and personnel contamination resulting from recombinant DNA research.

Note: The Laboratory Safety Monograph describes basic elements for developing specific procedures dealing with major spills of potentially hazardous materials in the laboratory, including information and references about decontamination and emergency plans. The NIH and the Centers for Disease Control and Prevention are available to provide consultation and direct assistance, if necessary, as posted in the Laboratory Safety Monograph. The institution shall cooperate with the state and local public health departments by reporting any significant research-related illness or accident that may be hazardous to the public health.

Section IV-B-2-b-(7). Reporting any significant problems with or violations of the NIH Guidelines and any significant research-related accidents or illnesses to the appropriate institutional official and NIH/ORDA within 30 days, unless the Institutional Biosafety Committee determines that a report has already been filed by the Principal Investigator. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-9838.

Section IV-B-2-b-(8). The Institutional Biosafety Committee may not authorize initiation of experiments which are not explicitly covered by the NIH Guidelines until NIH (with the advice of the RAC when required) establishes the containment requirement.

Section IV-B-2-b-(9). Performing such other functions as may be delegated to the Institutional Biosafety Committee under Section IV-B-2.

Section IV-B-3. Biological Safety Officer (BSO)

Section IV-B-3-a. The institution shall appoint a Biological Safety Officer if it engages in large scale research or production activities involving viable organisms containing recombinant DNA molecules.

Section IV-B-3-b. The institution shall appoint a Biological Safety Officer if it engages in recombinant DNA research at BL3 or BL4. The Biological Safety Officer shall be a member of the Institutional Biosafety Committee.

Section IV-B-3-c. The Biological Safety Officer's duties include, but are not be limited to:

Section IV-B-3-c-(1). Periodic inspections to ensure that laboratory standards are rigorously followed;

Section IV-B-3-c-(2). Reporting to the Institutional Biosafety Committee and the institution any significant problems, violations of the NIH Guidelines, and any significant research-related accidents or illnesses of which the Biological Safety Officer becomes aware unless the Biological Safety Officer determines that a report has already been filed by the Principal Investigator;

Section IV-B-3-c-(3). Developing emergency plans for handling accidental spills and personnel contamination and investigating laboratory accidents involving recombinant DNA research;

Section IV-B-3-c-(4). Providing advice on laboratory security;

Section IV-B-3-c-(5). Providing technical advice to Principal Investigators and the Institutional Biosafety Committee on research safety procedures.

Note: See the Laboratory Safety Monograph for additional information on the duties of the Biological Safety Officer.

Section IV-B-4. Principal Investigator (PI)

On behalf of the institution, the Principal Investigator is responsible for full compliance with the NIH Guidelines in the conduct of recombinant DNA research.

Section IV-B-4-a. General Responsibilities

As part of this general responsibility, the Principal Investigator shall:

Section IV-B-4-a-(1). Initiate or modify no recombinant DNA research which requires Institutional Biosafety Committee approval prior to initiation (see Sections III-A, III-B, and III-C) until that research or the proposed modification thereof has been approved by the Institutional Biosafety Committee and has met all other requirements of the NIH Guidelines;

Section IV-B-4-a-(2). Determine whether experiments are covered by Section III-D and that the appropriate procedures are followed;

Section IV-B-4-a-(3). Report any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable) within 30 days (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838);

Section IV-B-4-a-(4). Report any new information bearing on the NIH Guidelines to the Institutional Biosafety Committee and to NIH/ORDA (reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838);

Section IV-B-4-a-(5). Be adequately trained in good microbiological techniques;

Section IV-B-4-a-(6). Adhere to Institutional Biosafety Committee-approved emergency plans for handling accidental spills and personnel contamination; and

Section IV-B-4-a-(7). Comply with shipping requirements for recombinant DNA molecules (see Appendix H for shipping requirements and the Laboratory Safety Monograph for technical recommendations).

Section IV-B-4-b. Submissions by the Principal Investigator to the NIH/ORDA

The Principal Investigator shall:
Section IV-B-4-b-(1). Submit information to NIH/ORDA for certification of new host-vector systems;

Section IV-B-4-b-(2). Petition NIH/ORDA, with notice to the Institutional Biosafety Committee, for proposed exemptions to the NIH Guidelines;

Section IV-B-4-b-(3). Petition NIH/ORDA, with concurrence of the Institutional Biosafety Committee, for approval to conduct experiments specified in Sections III-A and III-B of the NIH Guidelines;

Section IV-B-4-b-(4). Petition NIH/ORDA for determination of containment for experiments requiring case-by-case review; and

Section IV-B-4-b-(5). Petition NIH/ORDA for determination of containment for experiments not covered by the NIH Guidelines.

Section IV-B-4-c. Submissions by the Principal Investigator to the Institutional Biosafety Committee

The Principal Investigator shall:

Section IV-B-4-c-(1). Make an initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines;

Section IV-B-4-c-(2). Select appropriate microbiological practices and laboratory techniques to be used for the research;

Section IV-B-4-c-(3). Submit the initial research protocol and any subsequent changes (e.g., changes in the source of DNA or host-vector system), if covered under Sections III-A, III-B, III-C, or III-D, to the Institutional Biosafety Committee for review and approval or disapproval; and

Section IV-B-4-c-(4). Remain in communication with the Institutional Biosafety Committee throughout the conduct of the project.

Section IV-B-4-d. Responsibilities of the Principal Investigator
Prior to Initiating Research The Principal Investigator shall:

Section IV-B-4-d-(1). Make available to all laboratory staff the protocols that describe the potential biohazards and the precautions to be taken;

Section IV-B-4-d-(2). Instruct and train laboratory staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents; and

Section IV-B-4-d-(3). Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or requested (e.g., vaccinations or serum collection).

Section IV-B-4-e. Responsibilities of the Principal Investigator During the Conduct of the Research

The Principal Investigator shall:

Section IV-B-4-e-(1). Supervise the safety performance of the laboratory staff to ensure that the required safety practices and techniques are employed;

Section IV-B-4-e-(2). Investigate and report any significant problems pertaining to the operation and implementation of containment practices and procedures in writing to the Biological Safety Officer (where applicable), Greenhouse/Animal Facility Director (where applicable), the Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable) (reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838);

Section IV-B-4-e-(3). Correct work errors and conditions that may result in the release of recombinant DNA materials; and

Section IV-B-4-e-(4). Ensure the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity and genotypic and phenotypic characteristics).

Section IV-C. Responsibilities of the National Institutes of Health (NIH)

Section IV-C-1. NIH Director

The NIH Director is responsible for: (i) establishing the NIH Guidelines, (ii) overseeing their implementation, and (iii) their final interpretation. The NIH Director has responsibilities under the NIH Guidelines that involve ORDA and the RAC. ORDA's responsibilities under the NIH Guidelines are administrative. Advice from the RAC is primarily scientific, technical, and ethical. In certain circumstances, there is specific opportunity for public comment with published response prior to final action.

Section IV-C-1-a. General Responsibilities

The NIH Director is responsible for:

Section IV-C-1-a-(1). Promulgating requirements as necessary to implement the NIH Guidelines;

Section IV-C-1-a-(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV-C-2 (RAC membership is specified in its charter and in Section IV-C-2); and

Section IV-C-1-a-(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV-C-3.

Section IV-C-1-b. Specific Responsibilities

In carrying out the responsibilities set forth in this section, the NIH Director, or a designee shall weigh each proposed action through appropriate analysis and consultation to determine whether it complies with the NIH Guidelines and presents no significant risk to health or the environment.

Section IV-C-1-b-(1). Major Actions

To execute Major Actions, the NIH Director shall seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the Notice of Meeting and Proposed Actions to the NIH Guidelines shall be published in the Federal Register at least 15 days before the RAC meeting (not applicable for Expedited Review single patient human gene transfer experiments considered under Appendix M-VI). The NIH Director's decision, at his/her discretion, may be published in the Federal Register for 15 days of comment before final action is taken. The NIH Director's final decision, along with responses to public comments, shall be published in the Federal Register. The RAC and Institutional Biosafety Committee Chairs shall be notified of the following decisions:

Section IV-C-1-b-(1)-(a). Changing containment levels for types of experiments that are specified in the NIH Guidelines when a Major Action is involved;

Section IV-C-1-b-(1)-(b). Assigning containment levels for types of experiments that are not explicitly considered in the NIH Guidelines when a Major Action is involved;

Section IV-C-1-b-(1)-(c). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from the NIH Guidelines because they consist entirely of DNA segments from species that exchange DNA by known physiological processes or otherwise do not present a significant risk to health or the environment;

Section IV-C-1-b-(1)-(d). Permitting experiments specified by Section III-A;

Section IV-C-1-b-(1)-(e). Certifying new host-vector systems with the exception of minor modifications of already certified systems (the standards and procedures for certification are described in Appendix I-II). Minor modifications constitute (e.g., those of minimal or no consequence to the properties relevant to containment); and

Section IV-C-1-b-(1)-(f). Adopting other changes in the NIH Guidelines.

Section IV-C-1-b-(2). Minor Actions

NIH/ORDA shall carry out certain functions as delegated to it by the NIH Director (see Section IV-C-3). Minor Actions (as determined by NIH/ORDA in consultation with the RAC Chair and one or more RAC members, as necessary) will be transmitted to the RAC and Institutional Biosafety Committee Chairs:

Section IV-C-1-b-(2)-(a). Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that qualify for the Accelerated Review process (see Section III-B-2);

Section IV-C-1-b-(2)-(b). Reviewing and approving minor changes to human gene transfer protocols under Section III-A-2 and III-B-2;

Section IV-C-1-b-(2)-(c). Changing containment levels for experiments that are specified in Section III;

Section IV-C-1-b-(2)-(d). Assigning containment levels for experiments not explicitly considered in the NIH Guidelines; and
Section IV-C-1-b-(2)-(e). Revising the Classification of Etiologic Agents for the purpose of these NIH Guidelines (see Section V-A).

Section IV-C-1-b-(2)-(f). Interpreting the NIH Guidelines for experiments to which the NIH Guidelines do not specifically assign containment levels;

Section IV-C-1-b-(2)-(g). Setting containment under Sections III-C-1-d and III-C-2-b;

Section IV-C-1-b-(2)-(h). Approving minor modifications of already certified host-vector systems (the standards and procedures for such modifications are described in Appendix I-II);

Section IV-C-1-b-(2)-(i). Decertifying already certified host-vector systems;

Section IV-C-1-b-(2)-(j). Adding new entries to the list of molecules toxic for vertebrates (see Appendix F); and

Section IV-C-1-b-(2)-(k). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV-C-1-b-(2)-(c).

Section IV-C-1-b-(3). The NIH Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

Section IV-C-2. Recombinant DNA Advisory Committee (RAC)

The RAC is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the DHHS Secretary, the DHHS Assistant Secretary for Health, and the NIH Director. The RAC consists of 25 members including the Chair, appointed by the DHHS Secretary or his/her designee, at least fourteen of whom are selected from authorities knowledgeable in the fields of molecular genetics, molecular biology, recombinant DNA research, or other scientific fields. At least six members of the RAC shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as non-voting members. Nominations for the RAC may be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

All meetings of the RAC shall be announced in the Federal Register, including tentative agenda items, 15 days before the meeting. Final agendas, if modified, shall be available at least 72 hours before the meeting. No item defined as a Major Action under Section IV-C-1-b-(1) may be added to an agenda following Federal Register publication.

The RAC shall be responsible for advising the NIH Director on the actions listed in Sections IV-C-1-b-(1).

Section IV-C-3. Office of Recombinant DNA Activities (ORDA)

ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH including institutions, Biological Safety Officers, Principal Investigators, Federal agencies, state and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the NIH Director, including those authorities described in Section IV-C-1-b-(2). ORDA's responsibilities include, but are not limited to the following:

Section IV-C-3-a. Reviewing and approving experiments in conjunction with ad hoc experts involving the cloning of genes encoding for toxin molecules that are lethal for vertebrates at an LD50 ≤100 nanograms per kilogram body weight in organisms other than Escherichia coli K-12 (see Section III-B-1 and Appendices F-I and F-II);

Section IV-C-3-b. Reviewing and approving certain experiments involving the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, in consultation with the RAC Chair and one or more RAC members, as necessary, that qualify for the Accelerated Review process (see Section III-B-2);

Section IV-C-3-c. Reviewing and approving minor changes to human gene transfer protocols approved under Sections III-A-2 and III-B-2, in consultation with the RAC Chair and one or more RAC members, as necessary;

Section IV-C-3-d. Reviewing and approving the membership of an institution's Institutional Biosafety Committee, and where it finds the Institutional Biosafety Committee meets the requirements set forth in Section IV-B-2 will give its approval to the Institutional Biosafety Committee membership;

Section IV-C-3-e. Publishing in the Federal Register:

Section IV-C-3-e-(1). Announcements of RAC meetings and agendas at least 15 days in advance (NOTE -- If the agenda for a RAC meeting is modified, ORDA shall make the revised agenda available to anyone upon request at least 72 hours in advance of the meeting);

Section IV-C-3-e-(2). Proposed Major Actions to the NIH Guidelines (see Section IV-C-1-b-(1)) at least 15 days prior to the RAC meeting;

Section IV-C-3-f. Serve as the focal point for data management of NIH-approved human gene transfer protocols approved under Sections III-A-2 and III-B-2 and registered with NIH/ORDA as required under Section III-C-7;

Section IV-C-3-g. Serve as the executive secretary of the RAC; and

Section IV-C-3-h. Maintain a list of Major and Minor Actions approved under Section III-A-2 and III-B-3 and a list of experiments registered with NIH/ORDA as described in Section III-C-7.

Section IV-C-4. Other NIH Components

Other NIH components shall be responsible for certifying maximum containment (BL4) facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary.

Section IV-D. Compliance with the NIH Guidelines

As a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the NIH Guidelines. The policies on noncompliance are as follows:

All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Non-compliance may result in: (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in: (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the institution.

Information concerning noncompliance with the NIH Guidelines may be brought forward by any person. It should be delivered to both NIH/ORDA and the relevant institution. The institution, generally through the Institutional Biosafety Committee, shall take appropriate action. The institution shall forward a complete report of the incident recommending any further action to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the NIH Guidelines, applicable DHHS and Public Health Service procedures shall govern.

Section IV-E. Voluntary Compliance

Section IV-E-1. Basic Policy

Individuals, corporations, and institutions not otherwise covered by the NIH Guidelines are encouraged to do so by following the standards and procedures set forth in Sections I through IV. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations and individuals who have no organizational affiliation. For purposes of complying with the NIH Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the NIH Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures are provided in Sections IV-E-2 through IV-E-5-b in order to address these concerns.

Section IV-E-2. Institutional Biosafety Committee Approval

It should be emphasized that employment of an Institutional Biosafety Committee member solely for purposes of membership on the Institutional Biosafety Committee does not itself make the member an institutionally affiliated member. Except for the unaffiliated members, a member of an Institutional Biosafety Committee for an institution not otherwise covered by the NIH Guidelines may participate in the review and approval of a project in which the member has a direct financial interest so long as the member has not been, and does not expect to be, engaged in the project. Section IV-B-2-a-(4) is modified to that extent for purposes of these institutions.

Section IV-E-3. Certification of Host-Vector Systems

A host-vector system may be proposed for certification by the NIH Director in accordance with the procedures set forth in Appendix I-II. In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section IV-E-5-a will be issued only after consultation with the institution as to the content of the notice.

Section IV-E-4. Requests for Exemptions and Approvals

Requests for exemptions or other approvals as required by the NIH Guidelines should be submitted based on the procedures set forth in Sections I through IV. In order to ensure protection for proprietary data, any public notice regarding a request for an exemption or other approval which is designated by the institution as proprietary under Section IV-E-5-a will be issued only after consultation with the institution as to the content of the notice.

Section IV-E-5. Protection of Proprietary Data

Section IV-E-5-a. General

In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial or financial information that is obtained from a person and that is privileged or confidential." Under 18 U.S.C. 1905, it is a criminal offense for an officer or employee of the U.S. or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, (or) processes...of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the RAC are "special Government employees."

In submitting to NIH for purposes of voluntary compliance with the NIH Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets, privileged, confidential, commercial, or financial information. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised except to the extent that earlier release in the judgment of the NIH Director is necessary to protect against an imminent hazard to the public or the environment.

Section IV-E-5-b. Presubmission Review

Any institution not otherwise covered by the NIH Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine if NIH will make all or part of the records available upon request under the Freedom of Information Act.

A request for presubmission review should be submitted to NIH/ORDA along with the records involved to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. These records shall be clearly marked as being the property of the institution on loan to NIH solely for the purpose of making a determination under the Freedom on Information Act. NIH/ORDA will seek a determination from the responsible official under DHHS regulations (45 Code of Federal Regulations, Part 5) as to whether the records involved, (or some portion) will be made available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from NIH/ORDA files, will be considered records of the institution and not NIH/ORDA, and will not be received as part of NIH/ORDA files. No copies will be made of such records.

NIH/ORDA will inform the institution of the DHHS Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of NIH/ORDA files. If the institution instructs NIH/ORDA to return the records, no copies or summaries of the records will be made or retained by DHHS, NIH, or ORDA. The DHHS Freedom of Information Officer's determination will represent that official's judgment at the time of the determination as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom on Information Act if at the time of the determination the records were in NIH/ORDA files and a request was received for such files under the Freedom of Information Act.

SECTION V. FOOTNOTES AND REFERENCES OF SECTIONS I THROUGH IV

Section V-A. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Services, Centers for Disease Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333. The NIH Director, with advice of the RAC, may revise the classification for the purposes of the NIH Guidelines (see Section IV-C-1-b-(2)-(e)). The revised list of organisms in each class is reprinted in Appendix B.

Section V-B. Section III describes a number of places where judgments are to be made. In all these cases, the Principal Investigator shall make the judgment on these matters as part of his/her responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the NIH Guidelines" (see Section IV-B-4-c-(1)). For cases falling under Sections III-A through III-D, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make an "independent assessment of the containment levels required by the NIH Guidelines for the proposed research" (see Section IV-B-2-b-(1)). The Institutional Biosafety Committee may refer specific cases to NIH/ORDA as part of NIH/ORDA's functions to "provide advice to all within and outside NIH" (see Section IV-C-3). NIH/ORDA may request advice from the RAC as part of the RAC's responsibility for "interpreting the NIH Guidelines for experiments to which the NIH Guidelines do not specifically assign containment levels" (see Section IV-C-1-b-(2)-(f)).

Section V-C. Laboratory Safety at the Centers for Disease Control, September 1974, U.S. Department of Health, Education, and Welfare Publication No. CDC 75-8118.

Section V-D. Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

Section V-E. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, October 1974, U.S. Department of Health, Education, and Welfare, Publication No. (NIH) 75-790.

Section V-F. National Institutes of Health Biohazards Safety Guide, 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740-00383.

Section V-G. A. Hellman, M. N. Oxman, and R. Pollack (eds.), 1973, Biohazards in Biological Research, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Section V-H. Furr, A. K., Handbook of Laboratory Safety, 2nd ed, The Chemical Rubber Co., Boca Raton, Florida, 1990.

Section V-I. American Public Health Association, Bodily, J. L., General Administration of the Laboratory, 6th ed., "Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections," New York, 1981.

Section V-J. H. M. Darlow, Safety in the Microbiological Laboratory, in J. R. Norris and D. W. Robbins (eds.), Methods in Microbiology, Academic Press, Inc, New York, New York, 1969, pp. 169-204.

Section V-K. C. M. Collins, E. G. Hartley, and R. Pilsworth, The Prevention of Laboratory Acquired Infection, Public Health Laboratory Service, Monograph Series No. 6, 1974.

Section V-L. Chatigny, M. A, "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W.W. Umbreit (ed.), Advances in Applied Microbiology, Academic Press, New York, New York, 1961, 3:131-192.

Section V-M. Design Criteria for Viral Oncology Research Facilities, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, DHEW Publication No. (NIH) 75-891, 1975.

Section V-N. Kuehne, R. W., Biological Containment Facility for Studying Infectious Disease, Appl. Microbiol. 26:239-243, 1973.

Section V-O. Runkle, R. B., and G. B. Phillips, Microbial Containment Control Facilities, Van Nostrand Reinhold, New York,
1969.

Section V-P. Chatigny, M. A., and D. I. Clinger, "Contamination Control in Aerobiology," in R. L. Dimmick and A. B. Akers (eds.), An Introduction to Experimental Aerobiology, John Wiley & Sons, New York, 1969, pp. 194-263.

Section V-Q. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

Section V-R. A U.S. Department of Agriculture permit is required for the importation, interstate movement, and release into the environment of certain organisms that are plant or animal pathogens, whether genetically engineered or not. Permits are required for veterinary biologics and for certain plants or microorganisms derived through genetic engineering using genetic sequences from plant pests (pathogens). Specific information about regulated organisms and procedures for obtaining a permit for regulated organisms may be obtained from the Director, Biotechnology, Biologics, and Environmental Protection, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, 6505 Belcrest Road, Room 850, Hyattsville, Maryland 20782, (301) 436-7601.

Section V-S. i.e., the total of all genomes within a family shall not exceed two-thirds of the genome.

Section V-T. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

Section V-U. Human studies in which the induction or enhancement of an immune response to a vector-encoded microbial immunogen is the major goal, such an immune response has been demonstrated in model systems, and the persistence of the vector-encoded immunogen is not expected, are not covered under Sections III-A-2, III-B-2, or III-B-3. Such studies may be initiated without RAC review and NIH approval if approved by another Federal agency.

Section V-V. For recombinant DNA experiments in which the intent is to modify stably the genome of cells of one or more human subjects (see Sections III-A-2, III-B-2, and III-B-3).

Section V-W. In accordance with accepted scientific and regulatory practices of the discipline of plant pathology, an exotic plant pathogen (e.g., virus, bacteria, or fungus) is one that is unknown to occur within the U.S. (see Section V-R). Determination of whether a pathogen has a potential for serious detrimental impact on managed (agricultural, forest, grassland) or natural ecosystems should be made by the Principal Investigator and the Institutional Biosafety Committee, in consultation with scientists knowledgeable of plant diseases, crops, and ecosystems in the geographic area of the research.

APPENDIX A. EXEMPTIONS UNDER SECTION III-E-5--SUBLISTS OF NATURAL EXCHANGERS

Certain specified recombinant DNA molecules that "consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent are exempt from these NIH Guidelines (see Section III-E-5). Institutional Biosafety Committee registration is not required for these exempt experiments. A list of such exchangers will be prepared and periodically revised by the NIH Director with advice from the RAC after appropriate notice and opportunity for public comment (see Section IV-C-1-b-(1)-(c)). See Appendices A-I through A-VI for a list of natural exchangers that are exempt from the NIH Guidelines." Section III-E-5 describes recombinant DNA molecules that are: (1) composed entirely of DNA segments from one or more of the organisms within a sublist, and (2) to be propagated in any of the organisms within a sublist (see Classification of Bergey's Manual of Determinative Bacteriology; 8th edition, R. E. Buchanan and N. E. Gibbons, editors, Williams and Wilkins Company; Baltimore, Maryland 1984). Although these experiments are exempt, it is recommended that they be performed at the appropriate biosafety level for the host or recombinant organism (see Biosafety in Microbiological and Biomedical Laboratories, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control, Atlanta, Georgia, and NIH Office of Biosafety, Bethesda, Maryland).

Appendix A-I. Sublist A

Genus Escherichia
Genus Shigella
Genus Salmonella - including Arizona
Genus Enterobacter
Genus Citrobacter - including Levinea
Genus Klebsiella - including oxytoca
Genus Erwinia
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, and Pseudomonas mendocina
Serratia marcescens
Yersinia enterocolitica

Appendix A-II. Sublist B

Bacillus subtilis
Bacillus licheniformis
Bacillus pumilus
Bacillus globigii
Bacillus niger
Bacillus nato
Bacillus amyloliquefaciens
Bacillus aterrimus

Appendix A-III. Sublist C

Streptomyces aureofaciens
Streptomyces rimosus
Streptomyces coelicolor

Appendix A-IV. Sublist D

Streptomyces griseus
Streptomyces cyaneus
Streptomyces venezuelae

Appendix A-V. Sublist E

One way transfer of Streptococcus mutans or Streptococcus lactis
DNA into Streptococcus sanguis

Appendix A-VI. Sublist F

Streptococcus sanguis
Streptococcus pneumoniae
Streptococcus faecalis
Streptococcus pyogenes
Streptococcus mutans

APPENDIX B. CLASSIFICATION OF ETIOLOGIC AGENTS AND ONCOGENIC VIRUSES ON THE BASIS OF HAZARD (See Appendix B-VI-A).

Appendix B-I. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes shall be considered Class 1 agents.

Appendix B-II. Class 2 Agents

Appendix B-II-A. Class 2 Bacterial Agents

Acinetobacter calcoaceticus
Actinobacillus - all species
Aeromonas hydrophila
Amycolata autotrophica
Arizona hinshawii - all serotypes
Bacillus anthracis
Bordetella - all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix insidiosa
Escherichia coli - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen
Haemophilus ducreyi, H. influenzae
Klebsiella - all species except oxytoca
Legionella pneumophila
Leptospira interrogans-all serotypes
Listeria - all species
Moraxella - all species
Mycobacteria - all species except those listed in Class 3
Mycobacterium avium
Mycoplasma - all species except Mycoplasma mycoides and
Mycoplasma agalactiae, which are in Class 5
Neisseria gonorrhoea, N. meningitides
Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis
Pasteurella - all species except those listed in Class 3
Rhodococcus equi
Salmonella - all species and all serotypes
Shigella-all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae, S. pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio cholerae, V. parahemolyticus
Yersinia enterocolitica

Appendix B-II-B. Class 2 Fungal Agents

Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides braziliensis

Appendix B-II-C. Class 2 Parasitic Agents

Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Schistosoma mansoni
Toxocara canis
Toxoplasma gondii
Trichinella spiralis
Trypanosoma cruzi

Appendix B-II-D. Class 2 Viral, Rickettsial, and Chlamydial Agents

Adenoviruses - human - all types
Cache Valley virus
Coronaviruses
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses - all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis viruses - associated antigen material
Herpesviruses - except Herpesvirus simiae (Monkey B virus) which is in Class 4
Influenza viruses - all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus - all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses - all types, wild and attenuated
Poxviruses - all types except Alastrim, Smallpox, and Whitepox which are Class 5 and Monkey pox which depending on experiments is in Class 3 or Class 4
Rabies virus - all strains except Rabies street virus which should be classified in Class 3
Reoviruses - all types
Respiratory syncytial virus
Rhinoviruses - all types
Rubella virus
Simian viruses - all types except Herpesvirus simiae (Monkey B virus) and Marburg virus which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vesicular stomatitis virus (see Appendix B-VI-B)
Vole rickettsia
Yellow fever virus, 17D vaccine strain

Appendix B-II-E. Class 2 Oncogenic Viruses (See Appendix B-VI-C)

Appendix B-II-E-1. Low-Risk Oncogenic Viruses

Adenovirus 7-Simian virus 40 (Ad7-SV40)
Adenovirus
Avian leukosis virus
Bovine leukemia virus
Bovine papilloma virus
Chick-embryo-lethal orphan (CELO) virus or fowl adenovirus 1
Dog sarcoma virus
Guinea pig herpes virus
Lucke (Frog) virus
Hamster leukemia virus
Marek's disease virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine leukemia virus
Murine sarcoma virus
Polyoma virus
Rat leukemia virus
Rous sarcoma virus
Shope fibroma virus
Shope papilloma virus
Simian virus 40 (SV-40)

Appendix B-II-E-2. Moderate-Risk Oncogenic Viruses

Adenovirus 2-Simian virus 40 (Ad2-SV40)
Epstein-Barr virus (EBV)
Feline leukemia virus (FeLV)
Feline sarcoma virus (FeSV)
Gibbon leukemia virus (GaLV)
Herpesvirus (HV) ateles
Herpesvirus (HV) saimiri
Simian sarcoma virus (SSV)-1
Yaba

Appendix B-III. Class 3 Agents

Appendix B-III-A. Class 3 Bacterial Agents

Bartonella - all species
Brucella - all species
Francisella tularensis
Mycobacterium bovis, M. tuberculosis
Pasteurella multocide type B -"buffalo" and other foreign virulent strains (see Appendix B-VI-B)
Pseudomonas mallei (see Appendix B-VI-B)
Pseudomonas pseudomallei (see Appendix B-VI-B)
Yersinia pestis

Appendix B-III-B. Class 3 Fungal Agents

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii

Appendix B-III-C. Class 3 Parasitic Agents

None

Appendix B-III-D. Class 3 Viral, Rickettsial, and Chlamydial Agents

Monkey pox virus - when used in vitro (see Appendix B-VI-D)
Arboviruses - all strains except those in Class 2 and 4. (Arboviruses indigenous to the United States are in Class 3 except those listed in Class 2. West Nile and Semliki Forest viruses may be classified up or down depending on the conditions of use and geographical location of the laboratory).
Dengue virus - when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Rickettsia - all species except Vole rickettsia when used for transmission or animal inoculation experiments
Yellow fever virus - wild, when used in vitro

Appendix B-IV. Class 4 Agents

Appendix B-IV-A. Class 4 Bacterial Agents

None

Appendix B-IV-B. Class 4 Fungal Agents

None

Appendix B-IV-C. Class 4 Parasitic Agents

None

Appendix B-IV-D. Class 4 Viral, Rickettsial, and Chlamydial
Agents

Ebola fever virus
Monkey pox virus - when used for transmission or animal inoculation experiments (see Appendix B-VI-D)
Hemorrhagic fever agents - including Crimean hemorrhagic fever, (Congo), Junin, and Machupo viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex - including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses
Venezuelan equine encephalitis virus, epidemic strains - when used for transmission or animal inoculation experiments
Yellow fever virus-wild - when used for transmission or animal inoculation experiments

Appendix B-V. Class 5 Agents (see Appendix B-VI-E)

Appendix B-V-A. Animal Disease Organisms which are Forbidden Entry into the United States by Law

Foot and mouth disease virus

Appendix B-V-B. Animal Disease Organisms and Vectors which are Forbidden Entry into the United States by U.S. Department of Agriculture Policy

African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Mycoplasma mycoides - contagious bovine pleuropneumonia
Mycoplasma agalactiae - contagious agalactia of sheep
Nairobi sheep disease virus
Newcastle disease virus - Asiatic strains
Rhinderpest virus
Rickettsia ruminatium - heart water
Rift valley fever virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Theileria parva - East Coast fever
Trypanosoma evansi
Trypanosoma vivax - Nagana
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Appendix B-V-C. Organisms which may not be Studied in the United States Except at Specified Facilities

Alastrim (see Appendix B-VI-D)
Small pox (see Appendix B-VI-D)
White pox (see Appendix B-VI-D)

Appendix B-VI. Footnotes and References of Appendix B

Appendix B-VI-A. The original reference for this classification was the publication Classification of Etiologic Agents on the Basis of Hazard, 4th edition, July 1974, U.S. DHHS, Public Health Service, Centers for Disease

Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these NIH Guidelines, this list has been revised by the NIH.
Appendix B-VI-B. A U.S. Department of Agriculture permit, required for import and interstate transport of pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products Office, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Appendix B-VI-C. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

Appendix B-VI-D. All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

Appendix B-VI-E. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

APPENDIX C. EXEMPTIONS UNDER SECTION III-E-6

Section III-E-6 states that exempt from these NIH Guidelines are "those that do not present a significant risk to health or the environment (see Section IV-C-1-b-(1)-(c)), as determined by the NIH Director, with the advice of the RAC, and following appropriate notice and opportunity for public comment. See Appendix C for other classes of experiments which are exempt from the NIH Guidelines." The following classes of experiments are exempt under Section III-E-6:

Appendix C-I. Recombinant DNA in Tissue Culture

Recombinant DNA molecules containing less than one-half of any eukaryotic viral genome (all viruses from a single family (see Appendix C-VI-D) being considered identical (see Appendix C-VI-E), that are propagated and maintained in cells in tissue culture are exempt from these NIH Guidelines with the exceptions listed in Appendix C-I-A.

Appendix C-I-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require specific RAC review and NIH and Institutional Biosafety Committee approval before initiation, (ii) experiments described in Section III-B which require NIH/ORDA and Institutional Biosafety Committee approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents, (iv) experiments involving the deliberate introduction of genes coding for the biosynthesis of molecules that are toxic for vertebrates (see Appendix F), and (v) whole plants regenerated from plant cells and tissue cultures are covered by the exemption provided they remain axenic cultures even though they differentiate into embryonic tissue and regenerate into plantlets.

Appendix C-II. Escherichia coli K-12 Host-Vector Systems

Experiments which use Escherichia coli K-12 host-vector systems, with the exception of those experiments listed in Appendix C-II-A, are exempt from the NIH Guidelines provided that: (i) the Escherichia coli host does not contain conjugation proficient plasmids or generalized transducing phages; or (ii) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids (see Appendix C-VI-B) shall be used as vectors. However, experiments involving the insertion into Escherichia coli K-12 of DNA from prokaryotes that exchange genetic information (see Appendix C-VI-C) with Escherichia coli may be performed with any Escherichia coli K-12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the Escherichia coli K-12 host may contain conjugation-proficient plasmids either autonomous or integrated, or generalized transducing phages. For these exempt laboratory experiments, Biosafety Level (BL) 1 physical containment conditions are recommended. For

large scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-II-A. Exceptions

The following categories of experiments are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH approval before initiation, (ii) experiments described in Section III-B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the cloning of toxin molecule genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-III. Saccharomyces Host-Vector Systems

Experiments involving Saccharomyces cerevisiae and Saccharomyces uvarum host-vector systems, with the exception of experiments listed in Appendix C-III-A, are exempt from the NIH Guidelines. For these exempt experiments, BL1 physical containment is recommended. For large scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if deemed necessary.

Appendix C-III-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH approval before initiation, (ii) experiments described in Section III-B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-IV. Bacillus subtilis or Bacillus licheniformis Host-Vector Systems

Any asporogenic Bacillus subtilis or asporogenic Bacillus licheniformis strain which does not revert to a spore-former with a frequency greater than 10-7 may be used for cloning DNA with the exception of those experiments listed in Appendix C-IV-A. For these exempt laboratory experiments, BL1 physical containment conditions are recommended. For large scale fermentation experiments, the appropriate physical containment conditions need be no greater than those for the host organism unmodified by recombinant DNA techniques; the Institutional Biosafety Committee can specify higher containment if it deems necessary.

Appendix C-IV-A. Exceptions

The following categories are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require Institutional Biosafety Committee approval, RAC review, and NIH approval before initiation, (ii) experiments described in Section III-B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-V. Extrachromosomal Elements of Gram Positive Organisms

Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C), propagated and maintained in organisms listed below are exempt from these NIH Guidelines.

Bacillus amyloliquefaciens
Bacillus amylosacchariticus
Bacillus anthracis
Bacillus aterrimus
Bacillus brevis
Bacillus cereus
Bacillus globigii
Bacillus licheniformis
Bacillus megaterium
Bacillus natto
Bacillus niger
Bacillus pumilus
Bacillus sphaericus
Bacillus stearothermophilis
Bacillus subtilis
Bacillus thuringiensis
Clostridium acetobutylicum
Lactobacillus casei
Listeria grayi
Listeria monocytogenes
Listeria murrayi
Pediococcus acidilactici
Pediococcus damnosus
Pediococcus pentosaceus
Staphylococcus aureus
Staphylcoccus carnosus
Staphylococcus epidermidis
Streptococcus agalactiae
Streptococcus anginosus
Streptococcus avium
Streptococcus cremoris
Streptococcus dorans
Streptococcus equisimilis
Streptococcus faecalis
Streptococcus ferus
Streptococcus lactis

Streptococcus ferns
Streptococcus mitior
Streptococcus mutans
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarious
Streptococcus sanguis
Streptococcus sobrinus
Streptococcus thermophylus

Appendix C-V-A. Exceptions

The following categories of experiments are not exempt from the NIH Guidelines: (i) experiments described in Section III-A which require Institutional Biosafety Committee, specific RAC review, and NIH approval before initiation, (ii) experiments described in Section III-B which require Institutional Biosafety Committee and NIH/ORDA approval before initiation, (iii) experiments involving DNA from Class 3, 4, or 5 organisms (see Appendix C-VI-A) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-C-2 with prior Institutional Biosafety Committee review and approval, (iv) large scale experiments (e.g., more than 10 liters of culture), and (v) experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F).

Appendix C-VI. Footnotes and References of Appendix C

Appendix C-VI-A. The original reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication Classification of Etiologic Agents on the Basis of Hazard, 4th Edition, July 1974, U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333.

Appendix C-VI-A-1. The NIH Director, with advice of the RAC, may revise the classification for the purposes of these NIH Guidelines (see Section IV-C-1-b-(2)-(d)). The revised list of organisms in each class is reprinted in Appendix B.

Appendix C-VI-B. A subset of non-conjugative plasmid vectors are poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

Appendix C-VI-C. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information.
Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section III-E-5.

Appendix C-VI-D. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews (ed.), Intervirology 12 (129-296), 1979.

Appendix C-VI-E. i.e., the total of all genomes within a Family shall not exceed one-half of the genome.

APPENDIX D. MAJOR ACTIONS TAKEN UNDER THE NIH GUIDELINES

Under Section IV-C-1-b-(1), the NIH Director may take certain actions with regard to the NIH Guidelines after the issues have been considered by the RAC. An updated list of these actions are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

APPENDIX E. CERTIFIED HOST-VECTOR SYSTEMS (SEE APPENDIX I)

While many experiments using Escherichia coli K-12, Saccharomyces cerevisiae, and Bacillus subtilis are currently exempt from the NIH Guidelines under Section III-E, some derivatives of these host-vector systems were previously classified as Host-Vector 1 Systems or Host-Vector 2 Systems. A listing of those systems follows:

Appendix E-I. Bacillus subtilis

Appendix E-I-A. Bacillus subtilis Host-Vector 1 Systems

The following plasmids are accepted as the vector components of certified B. subtilis systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. B. subtilis strains RUB 331 and BGSC 1S53 have been certified as the host component of Host-Vector 1 systems based on these plasmids.

Appendix E-I-B. Bacillus subtilis Host-Vector 2 Systems

The asporogenic mutant derivative of Bacillus subtilis, ASB 298, with the following plasmids as the vector component: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

Appendix E-II. Saccharomyces cerevisiae

Appendix E-II-A. Saccharomyces cerevisiae Host-Vector 2 Systems

The following sterile strains of Saccharomyces cerevisiae, all of which have the ste-VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E-III. Escherichia coli

Appendix E-III-A. Escherichia coli (EK2) Plasmid Systems

The Escherichia coli K-12 strain chi-1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR325, pBR327, pGL101, and pHB1. The following Escherichia coli/S. cerevisiae hybrid plasmids are certified as EK2 vectors when used in Escherichia coli chi-1776 or in the sterile yeast strains, SHY1, SHY2, SHY3, and SHY4: YIpI, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEP24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

Appendix E-III-B. Escherichia coli (EK2) Bacteriophage Systems

The following are certified EK2 systems based on bacteriophage lambda:

Vector	Host
gt WES B'	DP50supF
gt WES B*	DP50supF
gt ZJ vir B'	Escherichia coli K-12
gtALO· B	DP50supF
Charon 3A	DP50 or DP50supF
Charon 4A	DP50 or DP50supF
Charon 16A	DP50 or DP50supF
Charon 21A	DP50supF
Charon 23A	DP50 or DP50supF
Charon 24A	DP50 or DP50supF

Escherichia coli K-12 strains chi-2447 and chi-2281 are certified for use with lambda vectors that are certified for use with strain DP50 or DP50supF provided that the su-strain not be used as a propagation host.

Appendix E-IV. Neurospora crassa

Appendix E-IV-A. Neurospora crassa Host-Vector 1 Systems

The following specified strains of Neurospora crassa which have been modified to prevent aerial dispersion:

In1 (inositolless) strains 37102, 37401, 46316, 64001, and 89601. Csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).

Eas strain UCLA191 (an "easily wettable" mutant).

Appendix E-V. Streptomyces

Appendix E-V-A. Streptomyces Host-Vector 1 Systems

The following Streptomyces species: Streptomyces coelicolor, S. lividans, S. parvulus, and S. griseus. The following are accepted as vector components of certified Streptomyces Host-Vector 1 systems: Streptomyces plasmids SCP2, SLP1.2, pIJ101, actinophage phi C31, and their derivatives.

Appendix E-VI. Pseudomonas putida

Appendix E-VI-A. Pseudomonas putida Host-Vector 1 Systems

Pseudomonas putida strains KT2440 with plasmid vectors pKT262, pKT263, and pKT264.

APPENDIX F. CONTAINMENT CONDITIONS FOR CLONING OF GENES CODING FOR THE BIOSYNTHESIS OF MOLECULES TOXIC FOR VERTEBRATES

Appendix F-I. General Information

Appendix F specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The cloning of genes coding for molecules toxic for vertebrates that have an LD50 of <100 nanograms per kilograms body weight (e.g., microbial toxins such as the botulinum toxins, tetanus toxin, diphtheria toxin, Shigella dysenteriae neurotoxin) are covered under Section III-B-1 and require Institutional Biosafety Committee and NIH/ORDA approval before initiation. No specific restrictions shall apply to the cloning of genes if the protein specified by the gene has an LD50 ≥100 micrograms per kilograms of body weight. Experiments involving genes coding for toxin molecules with an LD50 of <100 micrograms per kilograms and >100 nanograms per kilograms body weight require Institutional Biosafety Committee approval and registration with NIH/ORDA prior to initiating the experiments. A list of toxin molecules classified as to LD50 is available from NIH/ORDA. Testing procedures for determining toxicity of toxin molecules not on the list are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. The results of such tests shall be forwarded to NIH/ORDA, which will consult with ad hoc experts, prior to inclusion of the molecules on the list (see Section IV-C-1-b-(2)-(e)).

Appendix F-II. Cloning of Toxin Molecule Genes in Escherichia coli K-12

Appendix F-II-A. Cloning of genes coding for molecules toxic for vertebrates that have an LD50 of >100 nanograms per kilograms and <1000 nanograms per kilograms body weight (e.g., abrin, Clostridium perfringens epsilon toxin) may proceed under Biosafety Level (BL) 2 + EK2 or BL3 + EK1 containment conditions.

Appendix F-II-B. Cloning of genes for the biosynthesis of molecules toxic for vertebrates that have an LD50 of >1 microgram per kilogram and <100 microgram per kilogram body weight may proceed under BL1 + EK1 containment conditions (e.g., Staphylococcus aureus alpha toxin, Staphylococcus aureus beta toxin, ricin, Pseudomonas aeruginosa exotoxin A, Bordetella pertussis toxin, the lethal factor of Bacillus anthracis, the Pasteurella pestis murine toxins, the oxygen-labile hemolysins such as streptolysin O, and certain neurotoxins present in snake venoms and other venoms).

Appendix F-II-C. Some enterotoxins are substantially more toxic when administered enterally than parenterally. The following enterotoxins shall be subject to BL1 + EK1 containment conditions: cholera toxin, the heat labile toxins of Escherichia coli, Klebsiella, and other related proteins that may be identified by neutralization with an antiserum monospecific for cholera toxin, and the heat stable toxins of Escherichia coli and of Yersinia enterocolitica.

Appendix F-III. Cloning of Toxic Molecule Genes in Organisms Other Than Escherichia coli K-12

Requests involving the cloning of genes coding for molecules toxic for vertebrates at an LD50 of <100 nanograms per kilogram body weight in host-vector systems other than Escherichia coli K-12 will be evaluated by NIH/ORDA in consultation with ad hoc toxin experts (see Sections III-B-1 and IV-C-1-b-(2)-(e)).

Appendix F-IV. Specific Approvals

An updated list of experiments involving the deliberate formation of recombinant DNA containing genes coding for toxins lethal for vertebrates at an LD50 of <100 nanograms per kilogram body weight is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

APPENDIX G. PHYSICAL CONTAINMENT

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1 through Biosafety Level 4. For large scale (over 10 liters) research or production, Appendix K supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q supersedes Appendix G. Appendix Q defines Biosafety Levels 1 through 4 - Animals.

Appendix G-I. Standard Practices and Training

The first principle of containment is strict adherence to good microbiological practices (see Appendices G-III-A through G-III-J). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV-B-1-e and IV-B-4-d). At a minimum, these instructions include training in aseptic techniques and in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents that are known or potential biohazards shall have an emergency plan that describes the procedures to be followed if an accident contaminates personnel or the environment. The Principal Investigator shall ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan (see Sections IV-B-4-d and IV-B-4-e). If a research group is working with a known pathogen for which there is an effective vaccine, the vaccine should be made available to all workers. Serological monitoring, when clearly appropriate, will be provided (see Section IV-B-1-f).

The Laboratory Safety Monograph (see Appendix G-III-O) and Biosafety in Microbiological and Biomedical Laboratories (see Appendix G-III-B) describe practices, equipment, and facilities in detail.

Appendix G-II. Physical Containment Levels

The objective of physical containment is to confine organisms containing recombinant DNA molecules and to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazard are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as BL1, BL2, BL3, and BL4 are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms (see Appendix G-III-B). The National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our BL2, BL3, and BL4 levels (see Appendix G-III-C).

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The NIH Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide BL3 and BL4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will be given by the NIH Director, with the advice of the RAC to other combinations which achieve an equivalent level of containment (see Section IV-C-1-b-(2)-(c)).

Appendix G-II-A. Biosafety Level 1 (BL1) (see Appendix G-III-M)

Appendix G-II-A-1. Standard Microbiological Practices (BL1)

Appendix G-II-A-1-a. Access to the laboratory is limited or restricted at the discretion of the Principal Investigator when experiments are in progress.

Appendix G-II-A-1-b. Work surfaces are decontaminated once a day and after any spill of viable material.

Appendix G-II-A-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-A-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-A-1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-A-1-f. Persons wash their hands: (i) after they handle materials involving organisms containing recombinant DNA molecules and animals, and (ii) before exiting the laboratory.

Appendix G-II-A-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-A-1-h. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules.

Appendix G-II-A-2. Special Practices (BL1)

Appendix G-II-A-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-A-2-b. An insect and rodent control program is in effect.

Appendix G-II-A-3. Containment Equipment (BL1)

Appendix G-II-A-3-a. Special containment equipment is generally not required for manipulations of agents assigned to BL1.

Appendix G-II-A-4. Laboratory Facilities (BL1)

Appendix G-II-A-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-A-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-A-4-c. Laboratory furniture is sturdy. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-A-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-A-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B. Biosafety Level 2 (BL2) (see Appendix G-III-N)

Appendix G-II-B-1. Standard Microbiological Practices (BL2)

Appendix G-II-B-1-a. Access to the laboratory is limited or restricted by the Principal Investigator when work with organisms containing recombinant DNA molecules is in progress.

Appendix G-II-B-1-b. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-B-1-c. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-B-1-d. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-B-1-e. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food may be stored in cabinets or refrigerators designated and used for this purpose only.

Appendix G-II-B-1-f. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules and animals, and (ii) when exiting the laboratory.

Appendix G-II-B-1-g. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-B-1-h. Experiments of lesser biohazard potential can be conducted concurrently in carefully demarcated areas of the same laboratory.

Appendix G-II-B-2. Special Practices (BL2)

Appendix G-II-B-2-a. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-B-2-b. The Principal Investigator limits access to the laboratory. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-B-2-c. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific entry requirements (e.g., immunization) may enter the laboratory or animal rooms.

Appendix G-II-B-2-d. When the organisms containing recombinant DNA molecules in use in the laboratory require special provisions for entry (e.g., vaccination), a hazard warning sign incorporating the universal biosafety symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

Appendix G-II-B-2-e. An insect and rodent control program is in effect.

Appendix G-II-B-2-f. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before exiting the laboratory for non-laboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.

Appendix G-II-B-2-g. Animals not involved in the work being performed are not permitted in the laboratory.

Appendix G-II-B-2-h. Special care is taken to avoid skin contamination with organisms containing recombinant DNA molecules; gloves should be worn when handling experimental animals and when skin contact with the agent is unavoidable.

Appendix G-II-B-2-i. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-B-2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably autoclaved, before discard or reuse.

Appendix G-II-B-2-k. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Institutional Biosafety Committee and NIH/ORDA. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix G-II-B-2-l. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix G-II-B-2-m. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.

Appendix G-II-B-3. Containment Equipment (BL 2)

Appendix G-II-B-3-a. Biological safety cabinets (Class I or II) (see Appendix G-III-L) or other appropriate personal protective or physical containment devices are used whenever:

Appendix G-II-B-3-a-(1). Procedures with a high potential for creating aerosols are conducted (see

Appendix G-III-O). These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of materials whose internal pressures may be different from ambient pressures, intranasal inoculation of animals, and harvesting infected tissues from animals or eggs.

Appendix G-II-B-3-a-(2). High concentrations or large volumes of organisms containing recombinant DNA molecules are used. Such materials may be centrifuged in the open laboratory if sealed beads or centrifuge safety cups are used and if they are opened only in a biological safety cabinet.

Appendix G-II-B-4. Laboratory Facilities (BL 2)

Appendix G-II-B-4-a. The laboratory is designed so that it can be easily cleaned.

Appendix G-II-B-4-b. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-B-4-c. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-B-4-d. Each laboratory contains a sink for hand washing.

Appendix G-II-B-4-e. If the laboratory has windows that open, they are fitted with fly screens.

Appendix G-II-B-4-f. An autoclave for decontaminating laboratory wastes is available.

Appendix G-II-C. Biosafety Level 3 (BL3) (see Appendix G-III-P)

Appendix G-II-C-1. Standard Microbiological Practices (BL3)

Appendix G-II-C-1-a. Work surfaces are decontaminated at least once a day and after any spill of viable material.

Appendix G-II-C-1-b. All contaminated liquid or solid wastes are decontaminated before disposal.

Appendix G-II-C-1-c. Mechanical pipetting devices are used; mouth pipetting is prohibited.

Appendix G-II-C-1-d. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.

Appendix G-II-C-1-e. Persons wash their hands: (i) after handling materials involving organisms containing recombinant DNA molecules, and handling animals, and (ii) when exiting the laboratory.

Appendix G-II-C-1-f. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-C-1-g. Persons under 16 years of age shall not enter the laboratory.

Appendix G-II-C-1-h. If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring BL3 level physical containment, they shall be conducted in accordance with all BL3 level laboratory practices.

Appendix G-II-C-2. Special Practices (BL3)

Appendix G-II-C-2-a. Laboratory doors are kept closed when experiments are in progress.

Appendix G-II-C-2-b. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable leak-proof container which is closed before being removed from the laboratory.

Appendix G-II-C-2-c. The Principal Investigator controls access to the laboratory and restricts access to persons whose presence is required for program or support purposes. The Principal Investigator has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.

Appendix G-II-C-2-d. The Principal Investigator establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., immunization), and who comply with all entry and exit procedures entering the laboratory or animal rooms.

Appendix G-II-C-2-e. When organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or containment module, a hazard warning sign incorporating the universal biosafety symbol is posted on all laboratory and animal room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the laboratory such as the need for immunizations, respirators, or other personal protective measures.

Appendix G-II-C-2-f. All activities involving organisms containing recombinant DNA molecules are conducted in biological safety cabinets or other physical containment devices within the containment module. No work in open vessels is conducted on the open bench.

Appendix G-II-C-2-g. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with organisms containing recombinant DNA molecules is finished. Plastic-backed paper toweling used on non-perforated work surfaces within biological safety cabinets facilitates clean-up.

Appendix G-II-C-2-h. An insect and rodent program is in effect.

Appendix G-II-C-2-i. Laboratory clothing that protects street clothing (e.g., solid front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated prior to laundering or disposal.

Appendix G-II-C-2-j. Special care is taken to avoid skin contamination with contaminated materials; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.

Appendix G-II-C-2-k. Molded surgical masks or respirators are worn in rooms containing experimental animals.

Appendix G-II-C-2-l. Animals and plants not related to the work being conducted are not permitted in the laboratory.

Appendix G-II-C-2-m. Laboratory animals held in a BL3 area shall be housed in partial-containment caging systems, such as Horsfall units (see Appendix G-III-K), open cages placed in ventilated enclosures, solid-wall and -bottom cages covered by filter bonnets or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet in radiation lamps and reflectors.

Note: Conventional caging systems may be used provided that all personnel wear appropriate personal protective devices. These protective devices shall include at a minimum wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.

Appendix G-II-C-2-n. All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.

Appendix G-II-C-2-o. Vacuum lines are protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix G-II-C-2-p. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix G-II-C-2-q. Spills and accidents which result in overt or potential exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/ORDA. Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Appropriate medical evaluation, surveillance, and treatment are provided and written records are maintained.

Appendix G-II-C-2-r. Baseline serum samples for all laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically depending on the agents handled or the function of the laboratory.

Appendix G-II-C-2-s. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read and follow the instructions on practices and procedures.

Appendix G-II-C-2-t. Alternative Selection of Containment Equipment (BL3)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified may be conducted in the BL3 laboratory using containment equipment specified for the BL4 level of physical containment. Alternative combination of containment safeguards are shown in Appendix G-Table 1.

Appendix G-II-C-3. Containment Equipment (BL3)

Appendix G-II-C-3-a. Biological safety cabinets (Class I, II, or III) (see Appendix G-III-L) or other appropriate combinations of personal protective or physical containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with organisms containing recombinant DNA molecules which pose a threat of aerosol exposure. These include: manipulation of cultures and of those clinical or environmental materials which may be a source of aerosols; the aerosol challenge of experimental animals; the harvesting of infected tissues or fluids from experimental animals and embryonate eggs; and the necropsy of experimental animals.

Appendix G-II-C-4. Laboratory Facilities (BL3)

Appendix G-II-C-4-a. The laboratory is separated from areas which are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high containment laboratory from access corridors or other laboratories or activities may be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility which requires passage through two sets of doors before entering the laboratory.

Appendix G-II-C-4-b. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.

Appendix G-II-C-4-c. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-C-4-d. Laboratory furniture is sturdy and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-C-4-e. Each laboratory contains a sink for hand washing. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.

Appendix G-II-C-4-f. Windows in the laboratory are closed and sealed.

Appendix G-II-C-4-g. Access doors to the laboratory or containment module are self-closing.

Appendix G-II-C-4-h. An autoclave for decontaminating laboratory wastes is available preferably within the laboratory.

Appendix G-II-C-4-i. A ducted exhaust air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the laboratory) is proper. The exhaust air from the laboratory room may be discharged to the outside without being filtered or otherwise treated.

Appendix G-II-C-4-j. The high efficiency particulate air/HEPA filtered exhaust air from Class I or Class II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every twelve months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G-III-L)) that avoids any interference with the air balance of the cabinets or building exhaust system.

Appendix G-II-D. Biosafety Level 4 (BL4)

Appendix G-II-D-1. Standard Microbiological Practices (BL4)

Appendix G-II-D-1-a. Work surfaces are decontaminated at least once a day and immediately after any spill of viable material.

Appendix G-II-D-1-b. Only mechanical pipetting devices are used.

Appendix G-II-D-1-c. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the laboratory.

Appendix G-II-D-1-d. All procedures are performed carefully to minimize the creation of aerosols.

Appendix G-II-D-2. Special Practices (BL4)

Appendix G-II-D-2-a. Biological materials to be removed from the Class III cabinets or from the maximum containment laboratory in a viable or intact state are transferred to a non-breakable, sealed primary container and then enclosed in a non-breakable, sealed secondary container which is removed from the facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose.

Appendix G-II-D-2-b. No materials, except for biological materials that are to remain in a viable or intact state, are removed from the maximum containment laboratory unless they have been autoclaved or decontaminated before exiting the facility. Equipment or material which might be damaged by high temperatures or steam is decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix G-II-D-2-c. Only persons whose presence in the facility or individual laboratory rooms is required for program or support purposes are authorized to enter. The supervisor has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory. Access to the facility is limited by means of secure, locked doors; accessibility is managed by the Principal Investigator, Biological Safety Officer, or other person responsible for the physical security of the facility. Before entering, persons are advised of the potential biohazards and instructed as to appropriate safeguards for ensuring their safety. Authorized persons comply with the instructions and all other applicable entry and exit procedures. A logbook signed by all personnel indicates the date and time of each entry and exit. Practical and effective protocols for emergency situations are established.

Appendix G-II-D-2-d. Personnel enter and exit the facility only through the clothing change and shower rooms. Personnel shower each time they exit the facility. Personnel use the air locks to enter or exit the laboratory only in an emergency.

Appendix G-II-D-2-e. Street clothing is removed in the outer clothing change room and kept there. Complete laboratory clothing (may be disposable), including undergarments, pants and shirts or jump suits, shoes, and gloves, is provided and used by all personnel entering the facility. Head covers are provided for personnel who do not wash their hair during the exit shower. When exiting the laboratory and before proceeding into the shower area, personnel remove their laboratory clothing and store it in a locker or hamper in the inner change room. Protective clothing shall be decontaminated prior to laundering or disposal.

Appendix G-II-D-2-f. When materials that contain organisms containing recombinant DNA molecules or experimental animals are present in the laboratory or animal rooms, a hazard warning sign incorporating the universal biosafety symbol is posted on all access doors. The sign identifies the agent, lists the name of the Principal Investigator or other responsible person(s), and indicates any special requirements for entering the area (e.g., the need for immunizations or respirators).

Appendix G-II-D-2-g. Supplies and materials needed in the facility are brought in by way of the double-doored autoclave, fumigation chamber, or airlock which is appropriately decontaminated between each use. After securing the outer doors, personnel within the facility retrieve the materials by opening the interior doors or the autoclave, fumigation chamber, or airlock. These doors are secured after materials are brought into the facility.

Appendix G-II-D-2-h. An insect and rodent control program is in effect.

Appendix G-II-D-2-i. Materials (e.g., plants, animals, and clothing) not related to the experiment being conducted are not permitted in the facility.

Appendix G-II-D-2-j. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle

units (i.e., needle is integral part of unit) are used for the injection or aspiration of fluids containing organisms that contain recombinant DNA molecules. Needles should not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe following use. The needle and syringe should be placed in a puncture-resistant container and decontaminated, preferably by autoclaving before discard or reuse. Whenever possible, cannulas are used instead of sharp needles (e.g., gavage).

Appendix G-II-D-2-k. A system is set up for reporting laboratory accidents, exposures, employee absenteeism, and for the medical surveillance of potential laboratory-associated illnesses. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, and NIH/ORDA. Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Written records are prepared and maintained. An essential adjunct to such a reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory associated illnesses.

Appendix G-II-D-2-l. Laboratory animals involved in experiments requiring BL4 level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems, such as Horsfall units (see Appendix G-III-K), open cages placed in ventilated enclosures, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors that are located in a specially designed area in which all personnel are required to wear one-piece positive pressure suits.

Appendix G-II-D-2-m. Alternative Selection of Containment Equipment (BL4)

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified may be conducted in the BL4 facility using containment equipment requirements specified for the BL3 level of physical containment. Alternative combinations of containment safeguards are shown in Appendix G-Table 1.

Appendix G-II-D-3. Containment Equipment (BL4)

Appendix G-II-D-3-a. All procedures within the facility with agents assigned to Biosafety Level 4 are conducted in the Class III biological safety cabinet or in Class I or II biological safety cabinets used in conjunction with one-piece positive pressure personnel suits ventilated by a life-support system.

Appendix G-II-D-4. Laboratory Facilities (BL4)

Appendix G-II-D-4-a. The maximum containment facility consists of either a separate building or a clearly demarcated and isolated zone within a building. Outer and inner change rooms separated by a shower are provided for personnel entering and exiting the facility. A double-doored autoclave, fumigation chamber, or ventilated airlock is provided for passage of those materials, supplies, or equipment which are not brought into the facility through the change room.

Appendix G-II-D-4-b. Walls, floors, and ceilings of the facility are constructed to form a sealed internal shell which facilitates fumigation and is animal and insect proof. The internal surfaces of this shell are resistant to liquids and chemicals, thus facilitating cleaning and decontamination of the area. All penetrations in these structures and surfaces are sealed. Any drains in the floors contain traps filled with a chemical disinfectant of demonstrated efficacy against the target agent, and they are connected directly to the liquid waste decontamination system. Sewer and other ventilation lines contain high efficiency particulate air/HEPA filters.

Appendix G-II-D-4-c. Internal facility appurtenances, such as light fixtures, air ducts, and utility pipes, are arranged to minimize the horizontal surface area on which dust can settle.

Appendix G-II-D-4-d. Bench tops have seamless surfaces which are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix G-II-D-4-e. Laboratory furniture is simple and of sturdy construction; and spaces between benches, cabinets, and equipment are accessible for cleaning.

Appendix G-II-D-4-f. A foot, elbow, or automatically operated hand washing sink is provided near the door of each laboratory room in the facility.

Appendix G-II-D-4-g. If there is a central vacuum system, it does not serve areas outside the facility. In-line high efficiency particulate air/HEPA filters are placed as near as practicable to each use point or service cock. Filters are installed to permit in-place decontamination and replacement. Other liquid and gas services to the facility are protected by devices that prevent back-flow.

Appendix G-II-D-4-h. If water fountains are provided, they are foot operated and are located in the facility corridors outside the laboratory. The water service to the fountain is not connected to the back-flow protected distribution system supplying water to the laboratory areas.

Appendix G-II-D-4-i. Access doors to the laboratory are self-closing and locking.

Appendix G-II-D-4-j. Any windows are breakage resistant.

Appendix G-II-D-4-k. A double-doored autoclave is provided for decontaminating materials passing out of the facility. The autoclave door which opens to the area external to the facility is sealed to the outer wall and automatically controlled so that the outside door can only be opened after the autoclave "sterilization" cycle has been completed.

Appendix G-II-D-4-l. A pass-through dunk tank, fumigation chamber, or an equivalent decontamination method is provided so that materials and equipment that cannot be decontaminated in the autoclave can be safely removed from the facility.

Appendix G-II-D-4-m. Liquid effluent from laboratory sinks, biological safety cabinets, floors, and autoclave chambers are decontaminated by heat treatment before being released from the maximum containment facility. Liquid wastes from shower rooms and toilets may be decontaminated with chemical disinfectants or by heat in the liquid waste decontamination system. The procedure used for heat decontamination of liquid wastes is evaluated mechanically and biologically by using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes from the shower room are decontaminated with chemical disinfectants, the chemical used is of demonstrated efficacy against the target or indicator microorganisms.

Appendix G-II-D-4-n. An individual supply and exhaust air ventilation system is provided. The system maintains pressure differentials and directional airflow as required to assure flows inward from areas outside of the facility toward areas of highest potential risk within the facility. Manometers are used to sense pressure differentials between adjacent areas maintained at different pressure levels. If a system malfunctions, the manometers sound an alarm. The supply and exhaust airflow is interlocked to assure inward (or zero) airflow at all times.

Appendix G-II-D-4-o. The exhaust air from the facility is filtered through high efficiency particulate air/HEPA filters and discharged to the outside so that it is dispersed away from occupied buildings and air intakes. Within the facility, the filters are located as near the laboratories as practicable in order to reduce the length of potentially contaminated air ducts. The filter chambers are designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Coarse filters and HEPA filters are provided to treat air supplied to the facility in order to increase the lifetime of the exhaust HEPA filters and to protect the supply air system should air pressures become unbalanced in the laboratory.

Appendix G-II-D-4-p. The treated exhaust air from Class I and II biological safety cabinets may be discharged into the laboratory room environment or the outside through the facility air exhaust system. If exhaust air from Class I or II biological safety cabinets is discharged into the laboratory the cabinets are tested and certified at six-month intervals. The exhaust air from Class III biological safety cabinets is discharged, without recirculation through two sets of high efficiency particulate air/HEPA filters in series, via the facility exhaust air system. If the treated exhaust air from any of these cabinets is discharged to the outside through the facility exhaust air system, it is connected to this system in a manner (e.g., thimble unit connection (see Appendix G-III-L)) that avoids any interference with the air balance of the cabinets or the facility exhaust air system.

Appendix G-II-D-4-q. A specially designed suit area may be provided in the facility. Personnel who enter this area shall wear a one-piece positive pressure suit that is ventilated by a life-support system. The life-support system includes alarms and emergency backup breathing air tanks. Entry to this area is through an airlock fitted with airtight doors. A chemical shower is provided to decontaminate the surface of the suit before the worker exits the area. The exhaust air from the suit area is filtered by two sets of high efficiency particulate air/HEPA filters installed in series. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source are provided. The air pressure within the suit area is greater than that of any adjacent area. Emergency lighting and communication systems are provided. All penetrations into the internal shell of the suit are sealed. A double-doored autoclave is provided for decontaminating waste materials to be removed from the suit areas.

APPENDIX G-TABLE 1. POSSIBLE ALTERNATE COMBINATIONS OF PHYSICAL AND BIOLOGICAL CONTAINMENT SAFEGUARDS

Classification of physical & biological containment	Alternate physical containment			Alternate biological containment
	Laboratory facilities	Laboratory practices	Laboratory equipment	
BL3/HV2	BL3	BL3	BL3	HV2
	BL3	BL3	BL4	HV1
BL3/HV1	BL3	BL3	BL3	HV1
	BL3	BL3	BL2	HV2
BL4/HV1	BL4	BL4	BL4	HV1
	BL4	BL4	BL3	HV2

BL-Biosafety Level
HV-HOst-Vector System

Appendix G-III. Footnotes and References of Appendix G.

Appendix G-III-A. Laboratory Safety at the Center for Disease Control, U.S. Department of Health, Education, and Welfare Publication No. CDC 75-8118, September 1974.

Appendix G-III-B. Biosafety in Microbiological and Biomedical Laboratories, 3rd edition, May 1993, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Atlanta, Georgia, and NIH, Bethesda, Maryland.

Appendix G-III-C. National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

Appendix G-III-D. National Institutes of Health Biohazards Safety Guide, U.S. Department of Health, Education, and Welfare, Public Health Service, NIH, U.S. Government Printing Office, Stock No. 1740-00383, 1974.

Appendix G-III-E. A. Hellman, M. N. Oxman, and R. Pollack (eds.), Biohazards in Biological Research, Cold Spring Harbor Laboratory 1973.

Appendix G-III-F. N. V. Steere (ed.), Handbook of Laboratory Safety, 2nd edition, The Chemical Rubber Co., Cleveland, Ohio, 1971.

Appendix G-III-G. Bodily, J. L, "General Administration of the Laboratory," H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic, and Parasitic Infections, American Public Health Association, New York, 1970, pp. 11-28.

Appendix G-III-H. Darlow, H. M. (1969). "Safety in the Microbiological Laboratory," in J. R. Norris and D. W. Robbins (eds.), Methods in Microbiology, Academic Press, Inc., New York, pp. 169-204.

Appendix G-III-I. The Prevention of Laboratory Acquired Infection, C. H. Collins, E. G. Hartley, and R. Pilsworth, Public Health Laboratory Service, Monograph Series No. 6, 1974.

Appendix G-III-J. Chatigny, M. A., "Protection Against Infection in the Microbiological Laboratory: Devices and Procedures," in W. W. Umbreit (ed.), Advances in Applied Microbiology, Academic Press, New York, New York, 1961, 3:131-192.

Appendix G-III-K. Horsfall, F. L. Jr., and J. H. Baner, Individual Isolation of Infected Animals in a Single Room, J. Bact., 1940, 40, 569-580.

Appendix G-III-L. Biological safety cabinets referred to in this section are classified as Class I, Class II, or Class III cabinets. A Class I is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high efficiency particulate air/HEPA filter. This cabinet is used in three operational modes: (i) with a full-width open front, (ii) with an installed front closure panel (having four 6-inch diameter openings) without gloves, and (iii) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A Class II cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for Class II cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A Class III cabinet is a closed-front ventilated cabinet of gas tight construction which provides the highest level of personnel protection of all biosafety safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment. National Sanitation Foundation Standard 49. 1976. Class II (Laminar Flow) Biohazard Cabinetry, Ann Arbor, Michigan.

Appendix G-III-M. Biosafety Level 1 is suitable for work involving agents of unknown or minimal potential hazard to laboratory personnel and the environment. The laboratory is not separated from the general traffic patterns in the building. Work is generally conducted on open bench tops. Special containment equipment is not required or generally used. Laboratory personnel have specific training in the procedures conducted in the laboratory and are supervised by a scientist with general training in microbiology or a related science (see Appendix G-III-B).

Appendix G-III-N. Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs in that: (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is

limited when work is being conducted; and (3) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment (see Appendix G-III-B).

Appendix G-III-O. Office of Research Safety, National Cancer Institute, and the Special Committee of Safety and Health Experts, Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research, NIH, Bethesda, Maryland 1978.

Appendix G-III-P. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is conducted with indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route. Laboratory personnel have specific training in handling pathogenic and potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal protective clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for BL3 (e.g., access zone, sealed penetrations, and directional airflow, etc.). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories where facility features satisfy BL2 recommendations provided the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for BL3 are rigorously followed. The decision to implement this modification of BL3 recommendations should be made only by the Principal Investigator.

APPENDIX H. SHIPMENT

Recombinant DNA molecules contained in an organism or in a viral genome shall be shipped under the applicable regulations of the U.S. Postal Service (39 Code of Federal Regulations, Part 3); the Public Health Service (42 Code of Federal Regulations, Part 72); the U.S. Department of Agriculture (9 Code of Federal Regulations, Subchapters D and E; 7 CFR, Part 340); and/or the U.S. Department of Transportation (49 Code of Federal Regulations, Parts 171-179).

Appendix H-I. Host organisms or viruses will be shipped as etiologic agents, regardless of whether they contain recombinant DNA, if they are regulated as human pathogens by the Public Health Service (42 Code of Federal Regulations, Part 72) or as animal pathogens or plant pests under the U.S. Department of Agriculture, Animal and Plant Health Inspection Service (Titles 9 and 7 Code of Federal Regulations, respectively).

Appendix H-II. Host organisms and viruses will be shipped as etiologic agents if they contain recombinant DNA when: (i) the recombinant DNA includes the complete genome of a host organism or virus regulated as a human or animal pathogen or a plant pest; or (ii) the recombinant DNA codes for a toxin or other factor directly involved in eliciting human, animal, or plant disease or inhibiting plant growth, and is carried on an expression vector or within the host chromosome and/or when the host organism contains a conjugation proficient plasmid or a generalized transducing phage; or (iii) the recombinant DNA comes from a host organism or virus regulated as a human or animal pathogen or as a plant pest and has not been adequately characterized to demonstrate that it does not code for a factor involved in eliciting human, animal, or plant disease.

Appendix H-III. Footnotes and References of Appendix H

For further information on shipping etiologic agents contact: (i) The Centers for Disease Control and Prevention, ATTN: Biohazards Control Office, 1600 Clifton Road, Atlanta, Georgia 30333, (404) 639-3883, FTS 236-3883; (ii) The U.S. Department of Transportation, ATTN: Office of Hazardous Materials Transportation, 400 7th Street, S.W., Washington, DC 20590, (202) 366-4545; or (iii) U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782; for Animal Pathogens call (301) 436-7885; for Plant Pests (301) 436-6799.

APPENDIX I. BIOLOGICAL CONTAINMENT (See Appendix E)

Appendix I-I. Levels of Biological Containment

In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment shall be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory, and (ii) transmission of the vector from the propagation host to other non-laboratory hosts. The following levels of biological containment (host-vector systems) for prokaryotes are established. Appendices I-I-A through I-II-B describe levels of biological containment (host-vector systems) for prokaryotes. Specific criteria will depend on the organisms to be used.

Appendix I-I-A. Host-Vector 1 Systems

Host-Vector 1 systems provide a moderate level of containment.
Specific Host-Vector 1 systems are:

Appendix I-I-A-1. Escherichia coli K-12 Host-Vector 1 Systems (EK1)

The host is always Escherichia coli K-12 or a derivative thereof, and the vectors include non-conjugative plasmids (e.g., pSC101, Co1E1, or derivatives thereof (see Appendices I-III-A through G) and variants of bacteriophage, such as lambda (see Appendices I-III-H through O). The Escherichia coli K-12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages.

Appendix I-I-A-2. Other Host-Vector 1 Systems

At a minimum, hosts and vectors shall be comparable in containment to Escherichia coli K-12 with a non-conjugative plasmid or bacteriophage vector. Appendix I-II describes the data to be considered and mechanism for approval of Host-Vector 1 systems.

Appendix I-I-B. Host-Vector 2 Systems

Host-Vector 2 Systems provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be <1/108 under specified conditions. Specific Host-Vector 2 systems are:

Appendix I-I-B-1. For Escherichia coli K-12 Host-Vector 2 systems (EK2) in which the vector is a plasmid, no more than 1/108 host cells shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

Appendix I-I-B-2. For Escherichia coli K-12 Host-Vector 2 systems (EK2) in which the vector is a phage, no more than 1/108 phage particles shall perpetuate a cloned DNA fragment under the specified non-permissive laboratory conditions designed to represent the natural environment, either as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation, or survival in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

Appendix I-II. Certification of Host-Vector Systems

Appendix I-II-A. Responsibility

Host-Vector 1 systems (other than Escherichia coli K-12) and Host-Vector 2 systems may not be designated as such until they have been certified by the NIH Director. Requests for certification of host-vector systems may be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Proposed host-vector systems will be reviewed by the RAC (see Section IV-C-1-b-(1)-(e)). Initial review will based on the construction, properties, and testing of the proposed host-vector system by a subcommittee composed of one or more RAC members and/or ad hoc experts. The RAC will evaluate the subcommittee's report and any other available information at the next scheduled RAC meeting. The NIH Director is responsible for certification of host-vector systems, following advice of the RAC. Minor modifications to existing host-vector systems (i.e., those that are of minimal or no consequence to the properties relevant to containment), may be certified by the NIH Director without prior RAC review (see Section IV-C-1-b-(2)-(h)). Once a host-vector system has been certified by the NIH Director, a notice of certification will be sent by NIH/ORDA to the applicant and to the Institutional Biosafety Committee Chairs. A list of all currently certified host-vector systems is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. The NIH Director may rescind the certification of a host-vector system (see Section IV-C-1-b-(2)-(i)). If certification is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system or use the clones at a higher level of physical containment level, unless NIH determines that the already constructed clones incorporate adequate biological containment. Certification of an host-vector system does not extend to modifications of either the host or vector component of that system. Such modified systems shall be independently certified by the NIH Director. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications to a certified host-vector system requires submission of complete testing data.

Appendix I-II-B. Data to be Submitted for Certification

Appendix I-II-B-1. Host-Vector 1 Systems Other than Escherichia coli K-12

The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) a description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction, survival, and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and the type of information is exchanged; and any relevant information about its pathogenicity or toxicity; (ii) a description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information; and (iii) a general description of the range of experiments contemplated with emphasis on the need for developing such an Host-Vector 1 system.

Appendix I-II-B-2. Host-Vector 2 Systems

Investigators planning to request Host-Vector 2 systems certification may obtain instructions from NIH/ORDA concerning data to be submitted (see Appendices I-III-N and O). In general, the following types of data are required: (i) description of construction steps with indication of source, properties, and manner of introduction of genetic traits; (ii) quantitative data on the stability of genetic traits that contribute to the containment of the system; (iii) data on the survival of the host-vector system under non-permissive laboratory conditions designed to represent the relevant natural environment; (iv) data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and non-permissive conditions; (v) data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation; and (vi) in some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals or one or more human subjects. Such in vivo data may be required to confirm the validity of predicting in vivo survival on the basis of in vitro experiments. Data shall be submitted 12 weeks prior to the RAC meeting at which such data will be considered by the Office of Recombinant DNA Activities, National Institutes of Health,

Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed Host Vector 2 systems prior to consideration of the system by the RAC and its subcommittee. Specific instructions concerning the submission of data for proposed Escherichia coli K-12 Host-Vector 2 system (EK2) involving either plasmids or bacteriophage in Escherichia coli K-12, are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Appendix I-III. Footnotes and References of Appendix I

Appendix I-III-A. Hersfield, V., H. W. Boyer, C. Yanofsky, M. A. Lovett, and D. R. Helinski, Plasmid Co1E1 as a Molecular Vehicle for Cloning and Amplification of DNA. Proc. Nat. Acad. Sci., 1974, 71, pp. 3455-3459.

Appendix I-III-B. Wensink, P. C., D. J. Finnegan, J. E. Donelson, and D. S. Hogness, A System for Mapping DNA Sequences in the Chromosomes of Drosophila Melanogaster. Cell, 1974, 3, pp. 315-335.

Appendix I-III-C. Tanaka, T., and B. Weisblum, Construction of a Colicin El-R Factor Composite Plasmid in Vitro: Means for Amplification of Deoxyribonucleic Acid. J. Bacteriol., 1975, 121, pp. 354-362.

Appendix I-III-D. Armstrong, K. A., V. Hershfield, and D. R. Helinski, Gene Cloning and Containment Properties of Plasmid Col E1 and Its Derivatives, Science, 1977, 196, pp. 172-174.

Appendix I-III-E. Bolivar, F., R. L. Rodriguez, M. C. Betlack, and H. W. Boyer, Construction and Characterization of New Cloning Vehicles: I. Ampicillin-Resistant Derivative of PMB9, Gene, 1977, 2, pp. 75-93.

Appendix I-III-F. Cohen, S. N., A. C. W. Chang, H. Boyer, and R. Helling. Construction of Biologically Functional Bacterial Plasmids in Vitro. Proc. Natl. Acad, Sci., 1973, 70, pp. 3240-3244.

Appendix I-III-G. Bolivar, F., R. L. Rodriguez, R. J. Greene, M. C.Batlack, H. L. Reyneker, H. W. Boyer, J. H. Cross, and S. Falkow, 1977, Construction and Characterization of New Cloning Vehicles II. A Multi-Purpose Cloning System, Gene, 1977, 2, pp. 95-113.

Appendix I-III-H. Thomas, M., J. R. Cameron, and R. W. Davis (1974). Viable Molecular Hybrids of Bacteriophage Lambda and Eukaryotic DNA. Proc. Nat. Acad. Sci., 1974, 71, pp. 4579-4583.

Appendix I-III-I. Murray, N. E., and K. Murray, Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments. Nature, 1974, 51, pp. 476-481.

Appendix I-III-J. Ramback, A., and P. Tiollais (1974). Bacteriophage Having EcoRI Endonuclease Sites Only in the Non-Essential Region of the Genome. Proc. Nat. Acad. Sci., 1974, 71, pp. 3927-3820.

Appendix I-III-K. Blattner, F. R., B. G. Williams, A. E. Bleche, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Gunwald, D. O. Kiefer, D. D. Moore, J. W. Shumm, E. L. Sheldon, and O. Smithies, Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning, Science 1977, 196, pp. 163-169.

Appendix I-III-L. Donoghue, D. J., and P. A. Sharp, An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance, Gene, 1977, 1, pp. 209-227.

Appendix I-III-M. Leder, P., D. Tiemeier and L. Enquist (1977), EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The gt WES System, Science, 1977, 196, pp. 175-177.

Appendix I-III-N. Skalka, A., Current Status of Coliphage AEK2 Vectors, Gene, 1978, 3, pp. 29-35.

Appendix I-III-O. Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein, Standardized Laboratory Tests for EK2 Certification, Gene, 1978, 3, pp. 36-38.

APPENDIX J. BIOTECHNOLOGY RESEARCH SUBCOMMITTEE

The National Science and Technology Council's Committee on Fundamental Science determined that a subcommittee should be continued to identify and coordinate Federal research efforts, identify research needs, stimulating international cooperation, and assess national and international policy issues concerning biotechnology sciences. The primary emphasis will be on scientific issues to increase the overall effectiveness and productivity of the Federal investment in biotechnology sciences, especially regarding issues which cut across agency boundaries. This subcommittee is called the Biotechnology Research Subcommittee.

Membership of the Biotechnology Research Subcommittee will include Federal agencies that support biotechnology research. Agencies represented are: U.S. Department of Agriculture, Department of Commerce, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Interior, Department of Justice, Department of State, Department of Veterans Affairs, Agency for International Development, Environmental Protection Agency, National Aeronautics and Space Administration, and National Science Foundation. The Biotechnology Research Subcommittee will function in an advisory capacity to the Committee on Fundamental Science, the Director of the Office of Science and Technology Policy, and the Executive Office of the President. The Biotechnology Research Subcommittee will review the scientific aspects of proposed regulations and guidelines as they are developed.

The primary responsibilities of the Biotechnology Research Subcommittee are to: (i) describe and review current Federal efforts in biotechnology research; (ii) identify and define the priority areas for future Federal biotechnology research, including areas needing greater emphasis, describing the role of each agency in those areas, and delineate where interagency cooperation would enhance progress in the biotechnology sciences, with an emphasis on integrated research efforts, where appropriate; (iii) assess major international efforts in the biotechnology sciences and develop mechanisms for international collaboration. For example, activities of the U.S.-European Community Task Force on Biotechnology have been coordinated through the Biotechnology Research Subcommittee; (iv) identify and review national and international policy issues (such as public education) associated with biotechnology; and (v) provide reviews, analyses, and recommendations to the Chairs of the Committee on Fundamental Science on scientific issues related to regulations and the applications of biotechnology research and biotechnology policies and issues.

In 1990, the Biotechnology Research Subcommittee replaced the Biotechnology Sciences Coordinating Committee. Both the Biotechnology Research Subcommittee and the Biotechnology Sciences Coordinating Committee previously functioned under the Federal Coordinating Council on Science, Engineering, and Technology (FCCSET). While regulatory issues became the primary focus of the Biotechnology Sciences Coordinating Committee, the Biotechnology Research Subcommittee focuses on scientific issues, although it will still provide scientific support for regulatory responsibilities.

APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT DNA MOLECULES

Appendix K specifies physical containment guidelines for large scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant DNA molecules. It shall apply to large scale research or production activities as specified in Section III-C-6. It is important to note that this appendix addresses only the biological hazard associated with organisms containing recombinant DNA. Other hazards accompanying the large scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this appendix. All provisions shall apply to large scale research or production activities with the following modifications: (i) Appendix K shall supersede Appendix G when quantities in excess of 10 liters of culture are involved in research or production. Appendix K-II applies to Good Large Scale Practice; (ii) the institution shall

appoint a Biological Safety Officer if it engages in large scale research or production activities involving viable organisms containing recombinant DNA molecules. The duties of the Biological Safety Officer shall include those specified in Section IV-B-3; (iii) the institution shall establish and maintain a health surveillance program for personnel engaged in large scale research or production activities involving viable organisms containing recombinant DNA molecules which require Biosafety Level (BL) 3 containment at the laboratory scale. The program shall include: preassignment and periodic physical and medical examinations; collection, maintenance, and analysis of serum specimens for monitoring serologic changes that may result from the employee's work experience; and provisions for the investigation of any serious, unusual, or extended illnesses of employees to determine possible occupational origin.

Appendix K-I. Selection of Physical Containment Levels

The selection of the physical containment level required for recombinant DNA research or production involving more than 10 liters of culture is based on the containment guidelines established in Section III. For purposes of large scale research or production, four physical containment levels are established. The four levels set containment conditions at those appropriate for the degree of hazard to health or the environment posed by the organism, judged by experience with similar organisms unmodified by recombinant DNA techniques and consistent with Good Large Scale Practice. The four biosafety levels of large scale physical containment are referred to as Good Large Scale Practice, BL1-Large Scale, BL2-Large Scale, and BL3-Large Scale. Good Large Scale Practice is recommended for large scale research or production involving viable, non-pathogenic, and non-toxigenic recombinant strains derived from host organisms that have an extended history of safe large scale use. Good Large Scale Practice is recommended for organisms such as those included in Appendix C which have built-in environmental limitations that permit optimum growth in the large scale setting but limited survival without adverse consequences in the environment. BL1-Large Scale is recommended for large scale research or production of viable organisms containing recombinant DNA molecules that require BL1 containment at the laboratory scale and that do not qualify for Good Large Scale Practice. BL2-Large Scale is recommended for large scale research or production of viable organisms containing recombinant DNA molecules that require BL2 containment at the laboratory scale. BL3-Large Scale is recommended for large scale research or production of viable organisms containing recombinant DNA molecules that require BL3 containment at the laboratory scale. No provisions are made for large scale research or production of viable organisms containing recombinant DNA molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

Appendix K-II. Good Large Scale Practice (GLSP)

Appendix K-II-A. Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters.

Appendix K-II-B. Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant DNA molecules are handled prudently and that the work place is kept clean and orderly.

Appendix K-II-C. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant DNA molecules. Eating, drinking, smoking, applying cosmetics, and mouth pipetting shall be prohibited in the work area.

Appendix K-II-D. Cultures of viable organisms containing recombinant DNA molecules shall be handled in facilities intended to safeguard health during work with microorganisms that do not require containment.

Appendix K-II-E. Discharges containing viable recombinant organisms shall be handled in accordance with applicable governmental environmental regulations.

Appendix K-II-F. Addition of materials to a system, sample collection, transfer of culture fluids within/between systems, and processing of culture fluids shall be conducted in a manner that maintains employee's exposure to viable organisms containing recombinant DNA molecules at a level that does not adversely affect the health and safety of employees.

Appendix K-II-G. The facility's emergency response plan shall include provisions for handling spills. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Appendix K-III. Biosafety Level 1 (BL1) - Large Scale

Appendix K-III-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-III-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to reduce the potential for escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G-II-A are met.

Appendix K-III-C. Culture fluids (except as allowed in Appendix K-III-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-III-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which minimizes the release of aerosols or contamination of exposed surfaces.

Appendix K-III-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to minimize the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K-III-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-III-G. Emergency plans required by Sections IV-B-2-b-(6) and IV-B-3-c-(3) shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K-IV. Biosafety Level 2 (BL2) - Large Scale

Appendix K-IV-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-IV-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessel used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system or other primary containment equipment provided all physical containment requirements specified in Appendix G-II-B are met.

Appendix K-IV-C. Culture fluids (except as allowed in Appendix K-IV-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organism that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-IV-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of cultures fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K-IV-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K-IV-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-IV-G. Rotating seals and other mechanical devices directly associated with a closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

Appendix K-IV-H. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing sensing devices that monitor the integrity of containment during operations.

Appendix K-IV-I. A closed system used for the propagation and growth of viable organisms containing the recombinant DNA molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K-IV-J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, and maintenance and in all documentation relating to use of this equipment for research or production activities involving viable organisms containing recombinant DNA molecules.

Appendix K-IV-K. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K-IV-L. Emergency plans required by Sections IV-B-2-b-(6) and IV-B-3-c-(3) shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K-V. Biosafety Level 3 (BL3) - Large Scale

Appendix K-V-A. Spills and accidents which result in overt exposures to organisms containing recombinant DNA molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-V-B. Cultures of viable organisms containing recombinant DNA molecules shall be handled in a closed system (e.g., closed vessels used for the propagation and growth of cultures) or other primary containment equipment (e.g., Class III biological safety cabinet containing a centrifuge used to process culture fluids) which is designed to prevent the escape of viable organisms. Volumes less than 10 liters may be handled outside of a closed system provided all physical containment requirements specified in Appendix G-II-C are met.

Appendix K-V-C. Culture fluids (except as allowed in Appendix K-V-D) shall not be removed from a closed system or other primary containment equipment unless the viable organisms containing recombinant DNA molecules have been inactivated by a validated inactivation procedure. A validated inactivation procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-V-D. Sample collection from a closed system, the addition of materials to a closed system, and the transfer of culture fluids from one closed system to another shall be conducted in a manner which prevents the release of aerosols or contamination of exposed surfaces.

Appendix K-V-E. Exhaust gases removed from a closed system or other primary containment equipment shall be treated by filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or by other equivalent procedures (e.g., incineration) to prevent the release of viable organisms containing recombinant DNA molecules to the environment.

Appendix K-V-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant DNA molecules shall not be opened for maintenance or other purposes unless it has been sterilized by a validated sterilization procedure. A validated sterilization procedure is one which has been demonstrated to be effective using the organisms that will serve as the host for propagating the recombinant DNA molecules.

Appendix K-V-G. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be operated so that the space above the culture level will be maintained at a pressure as low as possible, consistent with equipment design, in order to maintain the integrity of containment features.

Appendix K-V-H. Rotating seals and other mechanical devices directly associated with a closed system used to contain viable organisms containing recombinant DNA molecules shall be designed to prevent leakage or shall be fully enclosed in ventilated housings that are exhausted through filters which have efficiencies equivalent to high efficiency particulate air/HEPA filters or through other equivalent treatment devices.

Appendix K-V-I. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant DNA molecules shall include monitoring or sensing devices that monitor the integrity of containment during operations.

Appendix K-V-J. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant DNA molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant DNA molecules and following modification or replacement of essential containment features. Procedures and methods used in the testing shall be appropriate for the equipment design and for recovery and demonstration of the test organism. Records of tests and results shall be maintained on file.

Appendix K-V-K. A closed system used for the propagation and growth of viable organisms containing recombinant DNA molecules shall be permanently identified. This identification shall be used in all records reflecting testing, operation, maintenance, and use of this equipment for research production activities involving viable organisms containing recombinant DNA molecules.

Appendix K-V-L. The universal biosafety sign shall be posted on each closed system and primary containment equipment when used to contain viable organisms containing recombinant DNA molecules.

Appendix K-V-M. Emergency plans required by Sections IV-B-2-b-(6) and IV-B-3-c-(3) shall include methods and procedures for handling large losses of culture on an emergency basis.

Appendix K-V-N. Closed systems and other primary containment equipment used in handling cultures of viable organisms containing recombinant DNA molecules shall be located within a controlled area which meets the following requirements:

Appendix K-V-N-1. The controlled area shall have a separate entry area. The entry area shall be a double-doored space such as an air lock, anteroom, or change room that separates the controlled area from the balance of the facility.

Appendix K-V-N-2. The surfaces of walls, ceilings, and floors in the controlled area shall be such as to permit ready cleaning and decontamination.

Appendix K-V-N-3. Penetrations into the controlled area shall be sealed to permit liquid or vapor phase space decontamination.

Appendix K-V-N-4. All utilities and service or process piping and wiring entering the controlled area shall be protected against contamination.

Appendix K-V-N-5. Hand washing facilities equipped with foot, elbow, or automatically operated valves shall be located at each major work area and near each primary exit.

Appendix K-V-N-6. A shower facility shall be provided. This facility shall be located in close proximity to the controlled area.

Appendix K-V-N-7. The controlled area shall be designed to preclude release of culture fluids outside the controlled area in the event of an accidental spill or release from the closed systems or other primary containment equipment.

Appendix K-V-N-8. The controlled area shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential. If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the controlled area shall not be recirculated to other areas of the facility. The exhaust air from the controlled area may not be discharged to the outdoors without being high efficiency particulate air/HEPA filtered, subjected to thermal oxidation, or otherwise treated to prevent the release of viable organisms.

Appendix K-V-O. The following personnel and operational practices shall be required:

Appendix K-V-O-1. Personnel entry into the controlled area shall be through the entry area specified in Appendix K-V-N-1.

Appendix K-V-O-2. Persons entering the controlled area shall exchange or cover their personal clothing with work garments such as jump suits, laboratory coats, pants and shirts, head cover, and shoes or shoe covers. On exit from the controlled area the work clothing may be stored in a locker separate from that used for personal clothing or discarded for laundering. Clothing shall be decontaminated before laundering.

Appendix K-V-O-3. Entry into the controlled area during periods when work is in progress shall be restricted to those persons required to meet program or support needs. Prior to entry, all persons shall be informed of the operating practices, emergency procedures, and the nature of the work conducted.

Appendix K-V-O-4. Persons under 18 years of age shall not be permitted to enter the controlled area.

Appendix K-V-O-5. The universal biosafety sign shall be posted on entry doors to the controlled area and all internal doors when any work involving the organism is in progress. This includes periods when decontamination procedures are in progress. The sign posted on the entry doors to the controlled area shall include a statement of agents in use and personnel authorized to enter the controlled area.

Appendix K-V-O-6. The controlled area shall be kept neat and clean.

Appendix K-V-O-7. Eating, drinking, smoking, and storage of food are prohibited in the controlled area.

Appendix K-V-O-8. Animals and plants shall be excluded from the controlled area.

Appendix K-V-O-9. An effective insect and rodent control program shall be maintained.

Appendix K-V-O-10. Access doors to the controlled area shall be kept closed, except as necessary for access, while work is in progress. Serve doors leading directly outdoors shall be sealed and locked while work is in progress.

Appendix K-V-0-11. Persons shall wash their hands when exiting the controlled area.

Appendix K-V-O-12. Persons working in the controlled area shall be trained in emergency procedures.

Appendix K-V-O-13. Equipment and materials required for the management of accidents involving viable organisms containing recombinant DNA molecules shall be available in the controlled area.

Appendix K-V-O-14. The controlled area shall be decontaminated in accordance with established procedures following spills or other accidental release of viable organisms containing recombinant DNA molecules.

Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL)-Large Scale (LS) Practice (see Appendix K-VI-A)

Criterion (See Appendix K-VI-B)	GLSP	BL1-LS	BL2-LS	BL3-LS
1. Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures.	K-ll-A	G-l	G-l	G-l
2. Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees.	K-ll-B	G-lll	G-lll	G-lll
3. Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work.	K-ll-C	G-ll-A-1-h	G-ll-B-2-f	G-ll-C-2-i
4. Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place.	K-ll-C	G-ll-A-1-d G-ll-A-1-e	G-ll-B-1-d G-11-B-1-e	G-ll-C-1-c G-ll-C-1-d
5. Internal accident reporting.	K-ll-G	K-lll-A	K-IV-A	K-IV-A
6. Medical surveillance.	NR	NR	K-IV-A	K-V-A
7. Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment).	NR	K-lll-B	K-IV-B	K-V-B
8. Culture fluids not removed from a system until organisms are inactivated.	NR	K-lll-C	K-IV-C	K-V-C
9. Inactivation of waste solutions and materials with respect to their biohazard potential.	K-ll-E	K-lll-C	K-V-C	K-V-C
10. Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system.	Minimize Procedure K-ll-F	Minimize Engineer K-lll-B K-lll-D	Prevent Engineer K-IV-B K-IV-D	Prevent Engineer K-V-B K-V-D

Criterion (Appendix K-VI-B)	GLSP	BL1-LS	BL2-LS	BL3-LS
11. Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.	NR	Minimize K-lll-E	Prevent K-IV-E	Prevent K-V-E
12. Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure.	NR	K-lll-F	K-IV-F	K-V-F
13. Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features.	NR	NR	NR	K-V-G
14. Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.				
15. Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.	NR	NR	Prevent K-IV-G	Prevent K-V-H
16. Validated integrity testing of closed containment system.....	NR	NR	K-IV-H	K-V-I
17. Closed system to be permanently identified for record keeping purposes.	NR	NR	K-IV-l	K-V-J
18. Universal biosafety sign to be posted on each closed system.....	NR	NR	K-IV-J	K-V-K
19. Emergency plans required for handling large losses of cultures.	NR	NR	K-IV-K	K-V-L
20. Access to the work place...... 21. Requirements for controlled access area...............	K-ll-G	K-lll-G	K-IV-L	K-V-M
	NR	G-ll-A-1-a	G-ll-B-1-a	K-V-N
	NR	NR	NR	K-V-N&O

NR = not required

Appendix K-VI. Footnotes of Appendix K

Appendix K-VI-A. This table is derived from the text in Appendices G and K and is not to be used in lieu of Appendices G and K.

Appendix K-VI-B. The criteria in this grid address only the biological hazards associated with organisms containing recombinant DNA. Other hazards accompanying the large scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this grid.

Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K

Appendix K-VII-A. Accidental Release. An accidental release is the unintentional discharge of a microbiological agent (i.e., microorganism or virus) or eukaryotic cell due to a failure in the containment system.

Appendix K-VII-B. Biological Barrier. A biological barrier is an impediment (naturally occurring or introduced) to the infectivity and/or survival of a microbiological agent or eukaryotic cell once it has been released into the environment.

Appendix K-VII-C. Closed System. A closed system is one in which by its design and proper operation, prevents release of a microbiological agent or eukaryotic cell contained therein.

Appendix K-VII-D. Containment. Containment is the confinement of a microbiological agent or eukaryotic cell that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include: physical and biological barriers and inactivation using physical or chemical means.

Appendix K-VII-E. De minimis Release. De minimis release is the release of: (i) viable microbiological agents or eukaryotic cells that does not result in the establishment of disease in healthy people, plants, or animals; or (ii) in uncontrolled proliferation of any microbiological agents or eukaryotic cells.

Appendix K-VII-F. Disinfection. Disinfection is a process by which viable microbiological agents or eukaryotic cells are reduced to a level unlikely to produce disease in healthy people, plants, or animals.

Appendix K-VII-G. Good Large Scale Practice Organism. For an organism to qualify for Good Large Scale Practice consideration, it must meet the following criteria [Reference: Organization for Economic Cooperation and Development, Recombinant DNA Safety Considerations, 1987, p. 34-35]: (i) the host organism should be non-pathogenic, should not contain adventitious agents and should have an extended history of safe large scale use or have built-in environmental limitations that permit optimum growth in the large scale setting but limited survival without adverse consequences in the environment; (ii) the recombinant DNA-engineered organism should be non-pathogenic, should be as safe in the large scale setting as the host organism, and without adverse consequences in the environment; and (iii) the vector/insert should be well characterized and free from known harmful sequences; should be limited in size as much as possible to the DNA required to perform the intended function; should not increase the stability of the construct in the environment unless that is a requirement of the intended function; should be poorly mobilizable; and should not transfer any resistance markers to microorganisms unknown to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

Appendix K-VII-H. Inactivation. Inactivation is any process that destroys the ability of a specific microbiological agent or eukaryotic cell to self-replicate.

Appendix K-VII-I. Incidental Release. An incidental release is the discharge of a microbiological agent or eukaryotic cell from a containment system that is expected when the system is appropriately designed and properly operated and maintained.

Appendix K-VII-J. Minimization. Minimization is the design and operation of containment systems in order that any incidental release is a de minimis release.

Appendix K-VII-K. Pathogen. A pathogen is any microbiological agent or eukaryotic cell containing sufficient genetic information, which upon expression of such information, is capable of producing disease in healthy people, plants, or animals.

Appendix K-VII-L. Physical Barrier. A physical barrier is considered any equipment, facilities, or devices (e.g., fermentors, factories, filters, thermal oxidizers) which are designed to achieve containment.

Appendix K-VII-M. Release. Release is the discharge of a microbiological agent or eukaryotic cell from a containment system. Discharges can be incidental or accidental. Incidental releases are de minimis in nature; accidental releases may be de minimis in nature.

APPENDIX L. RELEASE INTO THE ENVIRONMENT OF CERTAIN PLANTS

Appendix L-I. General Information

Appendix L specifies conditions under which certain plants as specified below, may be approved for release into the environment. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, review by the RAC Plant Subcommittee, and specific approval by the NIH Director. Such experiments also require the approval of the Institutional Biosafety Committee before initiation.

Appendix L-II. Criteria Allowing Review by the RAC Plant Subcommittee Without the Requirement for Full RAC Review.

In consultation with the RAC Plant Subcommittee and without the requirement for full RAC review (Institutional Biosafety Committee review and approval is necessary), NIH/ORDA may approve the growing of plants containing recombinant DNA in the field under the following conditions: (i) the plant species is a cultivated crop of a genus that has no species known to be a noxious weed; (ii) the introduced DNA consists of well-characterized genes containing no sequences harmful to humans, animals, or plants; (iii) the vector consists of DNA from exempt host-vector systems (see Appendix C), from plants of the same or closely related species, from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants, from plants pathogens only if sequences resulting in production of disease symptoms have been deleted, or chimeric vectors constructed from sequences of exempt host-vector systems (see Appendix C) or from sequences from plant pathogens in which the disease symptoms have been deleted. The DNA may be introduced by any suitable method. If sequences resulting in production of disease symptoms are retained for purposes of introducing the DNA into the plant, greenhouse-grown plants must be shown to be free of such sequences before such plants, their derivatives, or seed can be used in field tests; (iv) plants are grown in controlled access fields under specified conditions appropriate for the plant under study and the geographical location. Such conditions should include provisions for using good cultural and pest control practices, for physical isolation from plants of the same species outside of the experimental plot in accordance with pollination characteristics of the species, and the prevention of plants containing recombinant DNA from becoming established in the environment. Review by the Institutional Biosafety Committee should include an appraisal by scientists knowledgeable of the crop, its production practices, and the local geographical conditions. Procedures for assessing alterations in and the spread of organisms containing recombinant DNA must be developed. The results of the outlined tests must be submitted for review and approval by the Institutional Biosafety Committee. Copies of such results must be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

APPENDIX M. POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF PROTOCOLS FOR THE TRANSFER OF RECOMBINANT DNA MOLECULES INTO THE GENOME OF ONE OR MORE HUMAN SUBJECTS (Points to Consider)

Appendix M applies to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from the NIH. Researchers not covered by the NIH Guidelines are encouraged to use Appendix M. Experiments in which recombinant DNA or DNA or RNA derived from recombinant DNA is introduced into one or more human subjects with the intent of stably modifying his/her genome are covered by Sections III-A-2, III-B-2, and III-B-3 (see Section V-U). Experiments in which recombinant DNA or DNA or RNA derived from recombinant DNA and that are not covered by Sections III-A-2, III-B-2, or III-B-3 and that are not considered exempt under Section V-U, are covered under Section III-C-7.

This document is intended to provide guidance in preparing proposals for NIH consideration under Sections III-A-2 and III-B-2. Section III-A-2 addresses Major Actions involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that have been determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to: (i) represent novel characteristics (e.g., target disease or vector), (ii) represent an uncertain degree of risk to human health or the environment, or (iii) contain information determined to require further public review. Proposals considered under Section III-A-2 will be reviewed by the RAC and approved by the NIH Director. RAC review of experiments considered under Section III-A-2 will follow publication of a precis of the proposal in the Federal Register and an opportunity for public comment. Section III-B-2 addresses Minor Actions involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects that have been determined by NIH/ORDA, in consultation with the RAC Chair and one or more RAC members, as necessary, to qualify for the Accelerated Review process. Proposals considered under Sections III-A-2 and III-B-2 will be on a case-by-case basis. A list of actions approved under Sections III-A-2 and III-B-2 involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects is available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. The list of actions to the NIH Guidelines involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects does not include experiments considered to be exempt from RAC and NIH/ORDA review under Section III-C-7.

Since the recombinant DNA or DNA or RNA derived from recombinant DNA is expected to be confined following transfer to one or more human subjects, no risk to public health or to the environment is expected. Nevertheless, Appendix M-I-B-4-b specifically asks the researchers to address this point.

This appendix will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out periodically as needed.

A proposal involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects will be considered by the RAC and/or NIH/ORDA only after the protocol has been approved by the local Institutional Biosafety Committee and Institutional Review Board in accordance with DHHS Regulations for the Federal Regulations for the Protection of Human Subjects (45 Code of Federal Regulations, Part 46). If a proposal involves children, special attention should be paid to subpart D of these DHHS regulations. The Institutional Review Board and Institutional Biosafety Committee may, at their discretion, condition their approval on further specific deliberation by the RAC and/or NIH/ORDA. Consideration of human gene transfer proposals by the RAC and/or NIH/ORDA may proceed simultaneously with review by other involved Federal agencies (see Appendix M-VII-A) provided that NIH/ORDA is notified of the simultaneous review. Meetings of the full RAC and its subcommittee will be open to the public except where trade secrets or proprietary information would be disclosed. The committee prefers that proposals submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. Public review of these protocols will serve to inform the public about the technical aspects of the proposals as well as the meaning and significance of the research.

The clinical application of recombinant DNA techniques raises two general kinds of questions: (i) the questions usually discussed by Institutional Review Boards in their review of any proposed research involving one or more human subjects; and (ii) broader issues. The first type of question is addressed principally in Appendix M-I of this document. Several broader issues are discussed throughout Appendix M.

Appendix M-I requests a description of the protocol with special attention to the short-term risks and benefits of the proposed research to the patient and to other people, the selection of patients, informed consent, privacy, and confidentiality. Appendix M-II addresses special issues pertaining to the free flow of information about the clinical trials. These issues lie outside the usual purview of Institutional Review Boards and reflect general public concerns about biomedical research. Appendix M-III summarizes guidelines for submission of human gene transfer protocols for RAC review. Appendix M-IV specifies reporting requirements. Appendix M-V describes the procedures for Accelerated Review of human gene transfer experiments. Appendix M-VI describes the procedures to be followed for Expedited Review of single patient human gene transfer experiments. Appendix M-VII contains the footnotes to Appendix M.

The RAC will not at present entertain proposals for germ-line alterations but will consider for approval protocols involving somatic cell gene transfer. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning gene into a patient's somatic cells. In germ-line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring.
The acceptability of human somatic cell gene therapy has been addressed in several public documents as well as in numerous academic studies. In November 1982, the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research published a report, Splicing Life, which resulted from a two-year process of public deliberations and hearing. Upon release of that report, a U.S. House of Representatives subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, Human Gene Therapy, which concluded: civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies. In light of this public support, the RAC is prepared to consider proposals for somatic cell gene therapy.

In its evaluation of proposals involving the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects, the RAC will consider whether the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of clinical investigations, namely, to protect the health and well-being of one or more human subjects being treated while at the same time gathering generalizable knowledge. Two possible undesirable consequences of the transfer of recombinant DNA would be unintentional: (i) vertical transmission of genetic changes from an individual to his/her offspring, or (ii) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, this document requests information that will enable the RAC and/or NIH/ORDA to assess the possibility that the proposed experiments will inadvertently affect reproductive cells or lead to infection of other people (e.g., medical personnel or relatives).

In recognition of the social concern that surrounds the subject of gene transfer, the RAC and NIH/ORDA will cooperate with other groups in assessing the possible long-term consequences of the transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into one or more human subjects and related laboratory and animal experiments in order to define appropriate human applications of this emerging technology.

Responses to Appendix M should be provided in the form of either written answers or references to specific sections of the protocol or its appendices. Principal Investigators should indicate points which are not applicable with a brief explanation. Principal Investigators submitting proposals that employ essentially the same vector

systems (or with minor variations), and/or that are based on the same preclinical testing as proposals previously reviewed by the RAC, may refer to preceding documents without having to rewrite such material.

Appendix M-I. Description of Proposal

Appendix M-I-A. Objectives and Rationale of the Proposed Research

State concisely the overall objectives and rationale of the proposed study. Provide information on the specific points that relate to whichever type of research is being proposed.

Appendix M-I-A-1. Use of Recombinant DNA for Therapeutic Purposes

For research in which recombinant DNA is transferred in order to treat a disease or disorder (e.g., genetic diseases, cancer, and metabolic diseases), the following questions should be addressed:

Appendix M-I-A-1-a. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

Appendix M-I-A-1-b. Describe the natural history and range of expression of the disease selected for treatment. What objective and/or quantitative measures of disease activity are available? In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene therapy?

Appendix M-I-A-1-c. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

Appendix M-I-A-1-d. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

Appendix M-I-A-2. Transfer of DNA for Other Purposes

Appendix M-I-A-2-a. Into what cells will the recombinant DNA be transferred? Why is the transfer of recombinant DNA necessary for the proposed research? What questions can be answered by using recombinant DNA?

Appendix M-I-A-2-b. What alternative methodologies exist? What are their relative advantages and disadvantages as compared to the use of recombinant DNA?

Appendix M-I-B. Research Design, Anticipated Risks and Benefits

Appendix M-I-B-1. Structure and Characteristics of the Biological System

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

Appendix M-I-B-1-a. What is the structure of the cloned DNA that will be used?

Appendix M-I-B-1-a-(1). Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

Appendix M-I-B-1-a-(2). What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)? From what source are these elements derived? Summarize what is currently known about the regulatory character of each element.

Appendix M-I-B-1-a-(3). Describe the steps used to derive the DNA construct.

Appendix M-I-B-1-b. What is the structure of the material that will be administered to the patient?

Appendix M-I-B-1-b-(1). Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells: (i) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests? (ii) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (for example, VL30 RNA, other nucleic acids, or proteins) or contaminating viruses (both replication-competent or replication-defective) or other organisms in the cells or serum used for preparation of the virus stock including any contaminants that may have biological effects? (iii) If co-cultivation is employed, what kinds of cells are being used for co-cultivation? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials? Specifically, what tests are being conducted to assess the material to be returned to the patient for the presence of live or killed donor cells or other non-vector materials (for example, VL30 sequences) originating from those cells? (iv) If methods other than those covered by Appendices M-I-B-1-b-(1)-(i) through (iii) are used to introduce new genetic information into target cells, what steps are being taken to detect and eliminate any contaminating materials? What are possible sources of contamination? What is the sensitivity of tests used to monitor contamination?

Appendix M-I-B-1-b-(2). Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

Appendix M-I-B-2. Preclinical Studies, Including Risk-Assessment Studies

Provide results that demonstrate the safety, efficacy, and feasibility of the proposed procedures using animal and/or cell culture model systems, and explain why the model(s) chosen is/are most appropriate.

Appendix M-I-B-2-a. Delivery System

Appendix M-I-B-2-a-(1). What cells are the intended target cells of recombinant DNA? What target cells are to be treated ex vivo and returned to the patient, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the target cells will incorporate the DNA?

Appendix M-I-B-2-a-(2). Is the delivery system efficient? What percentage of the target cells contain the added DNA?

Appendix M-I-B-2-a-(3). How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

Appendix M-I-B-2-a-(4). How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

Appendix M-I-B-2-b. Gene Transfer and Expression

Appendix M-I-B-2-b-(1). What animal and cultured cell models were used in laboratory studies to assess the in vivo and in vitro efficacy of the gene transfer system? In what ways are these models similar to and different from the proposed human treatment?

Appendix M-I-B-2-b-(2). What is the minimal level of gene transfer and/or expression that is estimated to be necessary for the gene transfer protocol to be successful in humans? How was this level determined?

Appendix M-I-B-2-b-(3). Explain in detail all results from animal and cultured cell model experiments which assess the effectiveness of the delivery system (see Appendix M-I-B-2-a) in achieving the minimally required level of gene transfer and expression (see Appendix M-I-B-2-b-(2)).

Appendix M-I-B-2-b-(4). To what extent is expression only from the desired gene (and not from the surrounding DNA)? To what extent does the insertion modify the expression of other genes?

Appendix M-I-B-2-b-(5). In what percentage of cells does expression from the added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene?

Appendix M-I-B-2-b-(6). Is the gene expressed in cells other than the target cells? If so, to what extent?

Appendix M-I-B-2-c. Retrovirus Delivery Systems

Appendix M-I-B-2-c-(1). What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

Appendix M-I-B-2-c-(2). How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement of recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

Appendix M-I-B-2-c-(3). What laboratory evidence is available concerning potential harmful effects of the transfer (e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses)? What steps will be taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

Appendix M-I-B-2-c-(4). Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ-line cells? What is the sensitivity of the analyses?

Appendix M-I-B-2-c-(5). Has a protocol similar to the one proposed for a clinical trial been conducted in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

Appendix M-I-B-2-d. Non-Retrovirus Delivery/Expression Systems. If a non-retroviral delivery system is used, what animal studies have been conducted to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ-line cells)? How long have the animals been studied after treatment? What safety studies have been conducted? (Include data about the level of sensitivity of such assays.)

Appendix M-I-B-3. Clinical Procedures, Including Patient Monitoring

Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study. Specifically:

Appendix M-I-B-3-a. Will cells (e.g., bone marrow cells) be removed from patients and treated ex vivo? If so, describe the type, number, and intervals at which these cells will be removed.

Appendix M-I-B-3-b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy)?

Appendix M-I-B-3-c. What treated cells (or vector/DNA combination) will be given to patients? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

Appendix M-I-B-3-d. How will it be determined that new gene sequences have been inserted into the patient's cells and if these sequences are being expressed? Are these cells limited to the intended target cell populations? How sensitive are these analyses?

Appendix M-I-B-3-e. What studies will be conducted to assess the presence and effects of the contaminants?

Appendix M-I-B-3-f. What are the clinical endpoints of the study? Are there objections and quantitative measurements to assess the natural history of the disease? Will such measurements be used in patient follow-up? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be conducted? How long will patient follow-up continue?

Appendix M-I-B-3-g. What are the major beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of deleterious consequences from the disease if recombinant DNA transfer is not used.

Appendix M-I-B-3-h. If a treated patient dies, what special post-mortem studies will be performed?

Appendix M-I-B-4. Public Health Considerations

Describe any potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically:

Appendix M-I-B-4-a. On what basis are potential public health benefits or hazards postulated?

Appendix M-I-B-4-b. Is there a significant possibility that the added DNA will spread from the patient to other persons or to the environment?

Appendix M-I-B-4-c. What precautions will be taken against such spread (e.g., patients sharing a room, health-care workers, or family members)?

Appendix M-I-B-4-d. What measures will be undertaken to mitigate the risks, if any, to public health?

Appendix M-I-B-4-e. In light of possible risks to offspring, including vertical transmission, will birth control measures be recommended to patients? Are such concerns applicable to health care personnel?

Appendix M-I-B-5. Qualifications of Investigators and Adequacy of Laboratory and Clinical Facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of recombinant DNA. Describe the laboratory and clinical facilities where the proposed study will be performed. Specifically:

Appendix M-I-B-5-a. What professional personnel (medical and nonmedical) will be involved in the proposed study and what is their relevant expertise? Provide a two-page curriculum vitae for each key professional person in biographical sketch format (see Appendix M-III-E).

Appendix M-I-B-5-b. At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period? What special arrangements will be made for the comfort and consideration of the patients. Will the research institution designate an ombudsman, patient care representative, or other individual to help protect the rights and welfare of the patient?

Appendix M-I-C. Selection of the Patients

Estimate the number of patients to be involved in the proposed study. Describe recruitment procedures and patient eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable. Specifically:

Appendix M-I-C-1. How many patients do you plan to involve in the proposed study?

Appendix M-I-C-2. How many eligible patients do you anticipate being able to identify each year?

Appendix M-I-C-3. What recruitment procedures do you plan to use?

Appendix M-I-C-4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

Appendix M-I-C-5. How will patients be selected if it is not possible to include all who desire to participate?

Appendix M-I-D. Informed Consent

Indicate how patients will be informed about the proposed study and how their consent will be solicited. The consent procedure should adhere to the requirements of DHHS regulations for the protection of human subjects (45 Code of Federal Regulations, Part 46). If the study involves pediatric or mentally handicapped patients, describe procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient. Areas of special concern include potential adverse effects, financial costs, privacy, long-term follow-up and post-mortem examination. When gene transfer is a procedure separate from a clinical protocol, Informed Consent documents shall be submitted for both the gene transfer and clinical protocols.

Appendix M-I-D-1. How will the major points covered in Appendices M-I-A through M-I-C be disclosed to potential participants in this study and/or parents or guardians in language that is understandable to them?

Appendix M-I-D-2. How will the innovative character and the theoretically possible adverse effects of the experiment be discussed with patients and/or parents or guardians? How will the potential adverse effects be compared with the consequences of the disease?

Appendix M-I-D-3. What explanation of the financial costs of the experiment, follow-up care, and any available alternatives will be provided to patients and/or parents or guardians?

Appendix M-I-D-4. How will patients and/or their parents or guardians be informed that the innovative character of the experiment may lead to great interest by the media in the research and in the treated patients?

Appendix M-I-D-5. How will the patients and/or their parents or guardians be informed about: (i) the irreversible consequences of some of the procedures performed? (ii) any adverse medical consequences that may occur if the subject(s) withdraws from the study once it has begun? (iii) expectations of willingness to cooperate in long-term follow-up? and (iv) expectations that permission to perform an autopsy will be granted in the event of a patient's death as a precondition for a patient's participation in the study? This stipulation is included because an accurate determination of the precise cause of a patient's death would be of vital importance to all future patients.

Appendix M-I-E. Privacy and Confidentiality

Indicate what measure will be taken to protect the privacy of patients and their families as well as to maintain the confidentiality of research data.

Appendix M-I-E-1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed.

Appendix M-I-E-2. What provision will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual patients?

Appendix M-II. Special Issues

Although the following issues are beyond the normal purview of local Institutional Review Boards, the RAC requests that Principal Investigators respond to Appendices M-II-A and M-II-B below:

Appendix M-II-A. What steps will be taken, consistent with Appendix M-I-E, to ensure that accurate and appropriate information is made available to the public with respect to such public concerns as may arise from the proposed study?

Appendix M-II-B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among Principal Investigators and clinicians concerning research methods and results?

Appendix M-III. Guidelines for the Submission of Human Gene Transfer Protocols

Appendices M-III-A through M-III-D and M-IV apply to human gene transfer protocols considered under Section III-A-2 and III-B-2. Appendices M-III-A, M-IV, and M-V apply to human gene transfer protocols considered under Section III-B-2.

Appendix M-III-A. Principal Investigator-Submitted Material

Principal Investigators should submit the following materials to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Appendix M-III-A-1. Written proposals shall be submitted in the following order: (1) scientific abstract--1 page; (2) non-technical abstract--1 page; (3) Institutional Biosafety Committee and Institutional Review Board approvals and their deliberations pertaining to your protocol; (4) Response to Points to Consider--5 pages (see Appendix M through M-III); (6) protocol--20 pages excluding appendices--approved by the local Institutional Biosafety Committee and Institutional Review Board); (7) Informed Consent document--approved by the Institutional Review Board; (8) appendices including tables, figures, and manuscripts; (9) curricula vitae--2 pages for each key professional person in biographical sketch format; and (10) an indication of other Federal agencies to which the protocol is being submitted for review.

Appendix M-III-A-2. When a proposal has been submitted previously, there should be a short section (≤ 200 words) immediately following the abstracts that summarizes the major revisions since the last review.

Appendix M-III-A-3. Data provided shall include: (i) a description of the elements in the vector, (ii) the source of that information, (iii) the method by which sequence data were compiled, and (iv) three 3½ inch diskettes with the vector sequence in ASCII format.

Appendix M-III-B. Time Frame for Submissions

Note: Time frames are applicable only to protocols that are determined by NIH/ORDA to require full RAC review and NIH Director approval. Time frames do not apply to Accelerated Review human gene transfer experiments (see Section III-B-2 or those that only require registration with NIH/ORDA (see Section III-C-7).

Appendix M-III-B-1. Written material from Principal Investigator shall be submitted ≥ 8 weeks before the RAC meeting at which it will be reviewed.

Appendix M-III-B-2. Written comments from the primary reviewers to the Principal Investigator shall be submitted ≥ 4 weeks before the RAC meeting at which it will be reviewed.

Appendix M-III-B-3. Written responses (including critical data in response to the primary reviewers' comments) shall be submitted by the Principal Investigator to NIH/ORDA ≥ 2 weeks before the RAC meeting.

Appendix M-III-C. Oral Responses to the RAC

Principal Investigators shall limit their oral responses to the RAC only to those questions that are raised during the meeting. Oral presentations of previously submitted material and/or critical data that was not submitted ≥ 2 weeks prior to the RAC meeting are prohibited.

Appendix M-III-D. Primary Reviewers' Responses

Appendix M-III-D-1. Primary Reviewers' Written Comments

The primary reviewers' written comments on a proposal should include the following:

Appendix M-III-D-1-a. Emphasize the issues related to gene marking, gene transfer, or gene therapy.

Appendix M-III-D-1-b. State explicitly whether the Points to Consider have been addressed satisfactorily.

Appendix M-III-D-1-c. Examine the scientific rationale, scientific context (relative to other proposals reviewed by the RAC), whether the preliminary in vitro and in vivo data were obtained in appropriate models and are sufficient, and whether questions related to safety, efficacy, and social/ethical context have been resolved.

Appendix M-III-D-1-d. Whenever possible, criticisms of Informed Consent documents should include written alternatives for suggested revisions for the RAC to consider.

Appendix M-III-D-1-e. Primary reviews should state whether the proposal is: (i) acceptable as written, (ii) expected to be acceptable with specific revisions or after satisfactory responses to specific questions raised on review, or (iii) unacceptable in its present form.

Appendix M-III-D-2. Oral Discussions by Primary Reviewers at the RAC Meeting

Appendix M-III-D-2-a. It should be possible for most primary reviewers to present their oral reviews in ≤ 5 minutes.

Appendix M-IV. Reporting Requirements

Appendix M-IV-A. Serious adverse effects of treatment should be reported immediately to the local Institutional Review Board, the NIH Office for Protection from Research Risks, and NIH/ORDA followed by the submission of a written report filed with each group. Reports submitted to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Appendix M-IV-B. Reports regarding the general progress of patients should be filed with both the local Institutional Review Board and NIH/ORDA within six months of the commencement of the experiment and at six-month intervals thereafter. These twice-yearly reports should continue for a sufficient period of time to allow observation of all major effects. In the event of a patient's death, a summary of the special post-mortem studies and statement of the cause of death should be submitted to the Institutional Review Board and NIH/ORDA, if available.

Appendix M-V. Procedures to the Followed for Accelerated Review of Human Gene Transfer Experiments by NIH/ORDA under Section III-B-2

Requests for Accelerated Review should be submitted to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-9838.

Appendix M-V-A. Human gene transfer experiments in this category must be in accordance with the provisions of Section III-B-2. If the human gene transfer protocol does not qualify for Accelerated Review (see Section III-B-2) as determined by NIH/ORDA, then the Principal Investigator must submit the experiment for full RAC review and NIH approval in accordance with Section III-A-2.

Appendix M-V-B. No protocol shall be considered without Institutional Biosafety Committee and Institutional Review Board approval.

Appendix M-V-C. At this time, all gene transfer protocols must be considered experimental.

Appendix M-V-D. Principal Investigators requesting Accelerated Review (see Section III-B-2), must submit the relevant documentation in accordance with Appendix M-III. NIH/ORDA will notify the Principal Investigator whether the proposed study qualifies for the Accelerated Review process. If NIH/ORDA determines that an experiment does not qualify for Accelerated Review process, the Principal Investigator must submit the proposal for full RAC review ≥ 8 weeks prior to the next scheduled RAC meeting.

Appendix M-V-E. It is expected that NIH/ORDA will consult with the RAC Chair and one or more RAC members, as necessary, when considering Accelerated Review human gene transfer protocols (see Section III-B-2).

Appendix M-V-F. The RAC Chair will provide a report on all human gene transfer protocols that have been approved by NIH/ORDA at the next regularly scheduled RAC meeting.

Appendix M-V-F-1. In accordance with Reporting Requirements (See Appendix M-IV), any adverse effects of the treatment should be reported immediately to the local Institutional Review Board, the NIH Office for Protection from Research Risks, and NIH/ORDA followed by the submission of a written report filed with each group. Reports submitted to NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838.

Appendix M-V-F-2. In accordance with Reporting Requirements (see Appendix M-IV), reports regarding the general progress of patients should be filed with both the local Institutional Review Board and NIH/ORDA within six months of the commencement of the experiment and at six-month intervals thereafter. In the event of a patient's death, a summary of the special post-mortem studies and statement of the cause of death should be submitted to the Institutional Review Board and NIH/ORDA, if available.

Appendix M-VI. Procedures to be Followed for Expedited Review of Single Patient Human Gene Transfer Experiments by NIH Director Under Section III-A-2

Requests for Expedited Review should be submitted to the Office of Recombinant DNA Activities, National Insitutes of Health, Bethesda, Maryland 20892, (301) 496-9838.

Appendix M-VI-A. A Principal Investigator submitting a request to the NIH/ORDA for Expedited Review of a single patient gene transfer protocol shall provide detailed information regarding the necessity of Expedited Review.

Appendix M-VI-B. No protocol shall be considered without relevant Institutional Biosafety Committee and Institutional Review Board approvals.

Appendix M-VI-C. At this time, all gene transfer protocols are considered experimental.

Appendix M-VI-D. Regardless of the method of review, the Points to Consider is the standard of review for all gene transfer protocols.

Appendix M-VI-E. Review of such protocols may include intramural NIH experts but must include extramural experts.

Appendix M-VI-F. The reviewers shall consider similarity of the new protocol to previously approved protocols.

Appendix M-VI-G. The NIH/ORDA shall report to the RAC following Expedited Review and include all of the materials on which the decision was based. The RAC shall formally review the protocol at its next scheduled meeting. Patient privacy shall be maintained.

Appendix M-VI-H. Protocols that are deferred or not approved by the RAC in its normal review process are not eligible for Expedited Review. No protocol shall have more than one patient approved under Expedited Review.

Appendix M-VI-I. As requested in the context of non-expedited review, none of the costs of the experimental protocol shall be borne by the patient or the patient's family.

Appendix M-VI-J. Data on all patients undergoing gene transfer shall be provided to the RAC within six months of the procedure.

Appendix M-VII. Footnotes of Appendix M

Appendix M-VII-A. The Food and Drug Administration has jurisdiction over products intended for use in human gene transfer clinical trials. For general information on the Food and Drug Administration's policies and regulatory requirements, see the Federal Register, Volume 51, pages 23309-23313, 1986.

Appendix M-VII-B. The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

APPENDIX P. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING PLANTS

Appendix P specifies physical and biological containment conditions and practices suitable to the greenhouse conduct of experiments involving recombinant DNA-containing plants, plant-associated microorganisms, and small animals. All provisions of the NIH Guidelines apply to plant research activities with the following modifications:

Appendix P shall supersede Appendix G when the research plants are of a size, number, or have growth requirements that preclude the use of containment conditions described in Appendix G. The plants covered in Appendix P include but are not limited to mosses, liverworts, macroscopic algae, and vascular plants including terrestrial crops, forest, and ornamental species.

Plant-associated microorganisms include viroids, virusoids, viruses, bacteria, fungi, protozoans, certain small algae, and microorganisms that have a benign or beneficial association with plants, such as certain Rhizobium species and microorganisms known to cause plant diseases. The appendix applies to microorganisms which are being modified with the objective of fostering an association with plants.

Plant-associated small animals include those arthropods that: (i) are in obligate association with plants, (ii) are plant pests, (iii) are plant pollinators, or (iv) transmit plant disease agents, as well as other small animals such as nematodes for which tests of biological properties necessitate the use of plants. Microorganisms associated with such small animals (e.g., pathogens or symbionts) are included.

The Institutional Biosafety Committee shall include at least one individual with expertise in plant, plant pathogen, or plant pest containment principles when experiments utilizing Appendix P require prior approval by the Institutional Biosafety Committee.

Appendix P-I. General Plant Biosafety Levels

Appendix P-I-A. The principal purpose of plant containment is to avoid the unintentional transmission of a recombinant DNA-containing plant genome, including nuclear or organelle hereditary material or release of recombinant DNA-derived organisms associated with plants.

Appendix P-I-B. The containment principles are based on the recognition that the organisms that are used pose no health threat to humans or higher animals (unless deliberately modified for that purpose), and that the containment conditions minimize the possibility of an unanticipated deleterious effect on organisms and ecosystems outside of the experimental facility, e.g., the inadvertent spread of a serious pathogen from a greenhouse to a local agricultural crop or the unintentional introduction and establishment of an organism in a new ecosystem.

Appendix P-I-C. Four biosafety levels, referred to as Biosafety Level (BL) 1 - Plants (P), BL2-P, BL3-P, and BL4-P, are established in Section II. The selection of containment levels required for research involving recombinant DNA molecules in plants or associated with plants is specified in Section III. These biosafety levels are described in Appendix P-II. This appendix describes greenhouse practices and special greenhouse facilities for physical containment.

Appendix P-I-D. BL1-P through BL4-P are designed to provide differential levels of biosafety for plants in the absence or presence of other experimental organisms that contain recombinant DNA. These biosafety levels, in conjunction with biological containment conditions described in Appendix P-III, provide flexible approaches to ensure the safe conduct of research.

Appendix P-I-E. For experiments in which plants are grown at the BL1 through BL4 laboratory settings, containment practices shall be followed as described in Appendix G. These containment practices include the use

of plant tissue culture rooms, growth chambers within laboratory facilities, or experiments performed on open benches. Additional biological containment practices should be added by the Greenhouse Director or Institutional Biosafety Committee as necessary (see Appendix P-III), if botanical reproductive structures are produced that have the potential of being released.

Appendix P-II. Physical Containment Levels

Appendix P-II-A. Biosafety Level 1 - Plants (BL1-P)

Appendix P-II-A-1. Standard Practices (BL1-P)

Appendix P-II-A-1-a. Greenhouse Access (BL1-P)

Appendix P-II-A-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, when experiments are in progress.

Appendix P-II-A-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL1-P greenhouse practices and procedures. All procedures shall be performed in accordance with accepted greenhouse practices that are appropriate to the experimental organism.

Appendix P-II-A-1-b. Records (BL1-P)

Appendix P-II-A-1-b-(1). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-A-1-c. Decontamination and Inactivation (BL1-P)

Appendix P-II-A-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-A-1-d. Control of Undesired Species and Motile Macroorganisms (BL1-P)

Appendix P-II-A-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens), by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-A-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-A-1-e. Concurrent Experiments Conducted in the Greenhouse (BL1-P)

Appendix P-II-A-1-e-(1). Experiments involving other organisms that require a containment level lower than BL1-P may be conducted in the greenhouse concurrently with experiments that require BL1-P containment, provided that all work is conducted in accordance with BL1-P greenhouse practices.

Appendix P-II-A-2. Facilities (BL1-P)

Appendix P-II-A-2-a. Definitions (BL1-P)

Appendix P-II-A-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-A-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area.

Appendix P-II-A-2-b. Greenhouse Design (BL1-P)

Appendix P-II-A-2-b-(1). The greenhouse floor may be composed of gravel or other porous material. At a minimum, impervious (e.g., concrete) walkways are recommended.

Appendix P-II-A-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to contain or exclude pollen, microorganisms, or small flying animals (e.g., arthropods and birds); however, screens are recommended.

Appendix P-II-B. Biosafety Level 2 - Plants (BL2-P)

Appendix P-II-B-1. Standard Practices (BL2-P)

Appendix P-II-B-1-a. Greenhouse Access (BL2-P)

Appendix P-II-B-1-a-(1). Access to the greenhouse shall be limited or restricted, at the discretion of the Greenhouse Director, to individuals directly involved with the experiments when they are in progress.

Appendix P-II-B-1-a-(2). Personnel shall be required to read and follow instructions on BL2-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-B-1-b. Records (BL2-P)

Appendix P-II-B-1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-B-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-B-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-B-1-c. Decontamination and Inactivation (BL2-P)

Appendix P-II-B-1-c-(1). Experimental organisms shall be rendered biologically inactive by appropriate methods before disposal outside of the greenhouse facility.

Appendix P-II-B-1-c-(2). Decontamination of run-off water is not necessarily required. If part of the greenhouse is composed of gravel or similar material, appropriate treatments should be made periodically to eliminate, or render inactive, any organisms potentially entrapped by the gravel.

Appendix P-II-B-1-d. Control of Undesired Species and Motile Macroorganisms (BL2-P)

Appendix P-II-B-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-B-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. If macroorganisms (e.g., flying arthropods or nematodes) are released within the greenhouse, precautions shall be taken to minimize escape from the greenhouse facility.

Appendix P-II-B-1-e. Concurrent Experiments Conducted in the Greenhouse (BL2-P)

Appendix P-II-B-1-e-(1). Experiments involving other organisms that require a containment level lower than BL2-P may be conducted in the greenhouse concurrently with experiments that require BL2-P containment provided that all work is conducted in accordance with BL2-P greenhouse practices.

Appendix P-II-B-1-f. Signs (BL2-P)

Appendix P-II-B-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-B-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-B-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-B-1-g. Transfer of Materials (BL2-P)

Appendix P-II-B-1-g-(1). Materials containing experimental microorganisms, which are brought into or removed from the greenhouse facility in a viable or intact state, shall be transferred in a closed non-breakable container.

Appendix P-II-B-1-h. Greenhouse Practices Manual (BL2-P)

Appendix P-II-B-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms.

Appendix P-II-B-2. Facilities (BL2-P)

Appendix P-II-B-2-a. Definitions (BL2-P)

Appendix P-II-B-2-a-(1). The term "greenhouse" refers to a structure with walls, a roof, and a floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-B-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas and is considered part of the confinement area.

Appendix P-II-B-2-b. Greenhouse Design (BL2-P)

Appendix P-II-B-2-b-(1). A greenhouse floor composed of an impervious material. Concrete is recommended, but gravel or other porous material under benches is acceptable unless propagules of experimental organisms are readily disseminated through soil. Soil beds are acceptable unless propagules of experimental organisms are readily disseminated through soil.

Appendix P-II-B-2-b-(2). Windows and other openings in the walls and roof of the greenhouse facility may be open for ventilation as needed for proper operation and do not require any special barrier to exclude pollen or microorganisms; however, screens are required to exclude small flying animals (e.g., arthropods and birds).

Appendix P-II-B-2-c. Autoclaves (BL2-P)

Appendix P-II-B-2-c-(1). An autoclave shall be available for the treatment of contaminated greenhouse materials.

Appendix P-II-B-2-d. Supply and Exhaust Air Ventilation Systems (BL2-P)

Appendix P-II-B-2-d-(1). If intake fans are used, measures shall be taken to minimize the ingress of arthropods. Louvers or fans shall be constructed such that they can only be opened when the fan is in operation.

Appendix P-II-B-2-e. Other (BL2-P)

Appendix P-II-B-2-e-(1). BL2-P greenhouse containment requirements may be satisfied by using a growth chamber or growth room within a building provided that the external physical structure limits access and escape of microorganisms and macroorganisms in a manner that satisfies the intent of the foregoing clauses.

Appendix P-II-C. Biosafety Level 3 - Plants (BL3-P)

Appendix P-II-C-1. Standard Practices (BL3-P)

Appendix P-II-C-1-a. Greenhouse Access (BL3-P)

Appendix P-II-C-1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility.

Appendix P-II-C-1-a-(2). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL3-P practices and procedures. All procedures shall be conducted in accordance with accepted greenhouse practices that are appropriate to the experimental organisms.

Appendix P-II-C-1-b. Records (BL3-P)

Appendix P-II-C-1-b-(1). A record shall be kept of experimental plants, microorganisms, or small animals that are brought into or removed from the greenhouse facility.

Appendix P-II-C-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-C-1-b-(3). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-C-1-c. Decontamination and Inactivation (BL3-P)

Appendix P-II-C-1-c-(1). All experimental materials shall be sterilized in an autoclave or rendered biologically inactive by appropriate methods before disposal, except those that are to remain in a viable or intact state for experimental purposes; including water that comes in contact with experimental microorganisms or with material exposed to such microorganisms, and contaminated equipment and supplies.

Appendix P-II-C-1-d. Control of Undesired Species and Motile Macroorganisms (BL3-P)

Appendix P-II-C-1-d-(1). A program shall be implemented to control undesired species (e.g., weed, rodent, or arthropod pests and pathogens) by methods appropriate to the organisms and in accordance with applicable state and Federal laws.

Appendix P-II-C-1-d-(2). Arthropods and other motile macroorganisms shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-C-1-e. Concurrent Experiments Conducted in the Greenhouse (BL3-P)

Appendix P-II-C-1-e-(1). Experiments involving organisms that require a containment level lower than BL3-P may be conducted in the greenhouse concurrently with experiments that require BL3-P containment provided that all work is conducted in accordance with BL3-P greenhouse practices.

Appendix P-II-C-1-f. Signs (BL3-P)

Appendix P-II-C-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-C-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence should be indicated on a sign posted on the greenhouse access doors.

Appendix P-II-C-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-C-1-g. Transfer of Materials (BL3-P)

Appendix P-II-C-1-g-(1). Experimental materials that are brought into or removed from the greenhouse facility in a viable or intact state shall be transferred to a non-breakable sealed secondary container. At the time of transfer, if the same plant species, host, or vector are present within the effective dissemination distance of propagules of the experimental organism, the surface of the secondary container shall be decontaminated. Decontamination may be accomplished by passage through a chemical disinfectant or fumigation chamber or by an alternative procedure that has demonstrated effective inactivation of the experimental organism.

Appendix P-II-C-1-h. Greenhouse Practices Manual (BL3-P)

Appendix P-II-C-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall: (i) advise personnel of the potential consequences if such practices are not followed, and (ii) outline contingency plans to be implemented in the event of the unintentional release of organisms with recognized potential for serious detrimental impact.

Appendix P-II-C-1-i. Protective Clothing (BL3-P)

Appendix P-II-C-1-i-(1). Disposable clothing (e.g., solid front or wrap-around gowns, scrub suits, or other appropriate clothing) shall be worn in the greenhouse if deemed necessary by the Greenhouse Director because of potential dissemination of the experimental microorganisms.

Appendix P-II-C-1-i-(2). Protective clothing shall be removed before exiting the greenhouse and decontaminated prior to laundering or disposal.

Appendix P-II-C-1-j. Other (BL3-P)

Appendix P-II-C-1-j-(1). Personnel are required to thoroughly wash their hands upon exiting the greenhouse.

Appendix P-II-C-1-j-(2). All procedures shall be performed carefully to minimize the creation of aerosols and excessive splashing of potting material/soil during watering, transplanting, and all experimental manipulations.

Appendix P-II-C-2. Facilities (BL3-P)

Appendix P-II-C-2-a. Definitions (BL3-P)

Appendix P-II-C-2-a-(1). The term "greenhouse" refers to a structure with walls, roof, and floor designed and used principally for growing plants in a controlled and protected environment. The walls and roof are usually constructed of transparent or translucent material to allow passage of sunlight for plant growth.

Appendix P-II-C-2-a-(2). The term "greenhouse facility" includes the actual greenhouse rooms or compartments for growing plants, including all immediately contiguous hallways and head-house areas, and is considered part of the confinement area. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse.

Appendix P-II-C-2-b. Greenhouse Design (BL3-P)

Appendix P-II-C-2-b-(1). The greenhouse floor shall be composed of concrete or other impervious material with provision for collection and decontamination of liquid run-off.

Appendix P-II-C-2-b-(2). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-C-2-b-(3). The greenhouse shall be a closed self-contained structure with a continuous covering that is separated from areas that are open to unrestricted traffic flow. The minimum requirement for greenhouse entry shall be passage through two sets of self-closing locking doors.

Appendix P-II-C-2-b-(4). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-C-2-b-(5). Internal walls, ceilings, and floors shall be resistant to penetration by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-C-2-b-(6). Bench tops and other work surfaces should have seamless surfaces that are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-C-2-b-(7). The greenhouse contains a foot, elbow, or automatically operated sink, which is located near the exit door for hand washing.

Appendix P-II-C-2-c. Autoclaves (BL3-P)

Appendix P-II-C-2-c-(1). An autoclave shall be available for decontaminating materials within the greenhouse facility. A double-door autoclave is recommended (not required) for the decontamination of materials passing out of the greenhouse facility.

Appendix P-II-C-2-d. Supply and Exhaust Air Ventilation Systems (BL3-P)

Appendix P-II-C-2-d-(1). An individual supply and exhaust air ventilation system shall be provided. The system maintains pressure differentials and directional airflow, as required, to assure inward (or zero) airflow from areas outside of the greenhouse.

Appendix P-II-C-2-d-(2). The exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air-HEPA filters and discharged to the outside. The filter chambers shall be designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. Air filters shall be 80-85% average efficiency by the American Society of Heating, Refrigerating, and Air Conditioning Engineers (ASHRAE) Standard 52-68 test method using atmosphere dust. Air supply fans shall be equipped with a back-flow damper that closes when the air supply fan is off. Alternatively, a HEPA filter may be used on the air supply system instead of the filters and damper. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times.

Appendix P-II-C-2-e. Other (BL3-P)

Appendix P-II-C-2-e-(1). BL3-P greenhouse containment requirements may be satisfied using a growth chamber or growth room within a building provided that the location, access, airflow patterns, and provisions for decontamination of experimental materials and supplies meet the intent of the foregoing clauses.

Appendix P-II-C-2-e-(2). Vacuum lines shall be protected with high efficiency particulate air/HEPA or equivalent filters and liquid disinfectant traps.

Appendix P-II-D. Biosafety Level 4 - Plants (BL4-P)

Appendix P-II-D-1. Standard Practices (BL4-P)

Appendix P-II-D-1-a. Greenhouse Access (BL4-P)

Appendix P-II-D-1-a-(1). Authorized entry into the greenhouse shall be restricted to individuals who are required for program or support purposes. The Greenhouse Director shall be responsible for assessing each circumstance and determining those individuals who are authorized to enter the greenhouse facility or work in the greenhouse during experiments.

Appendix P-II-D-1-a-(2). Access shall be managed by the Greenhouse Director, Biological Safety Officer, or other individual responsible for physical security of the greenhouse facility; and access limited by means of secure, locked doors.

Appendix P-II-D-1-a-(3). Prior to entering, individuals shall be advised of the potential environmental hazards and instructed on appropriate safeguards for ensuring environmental safety. Individuals authorized to enter the greenhouse facility shall comply with the instructions and all other applicable entry/exit procedures.

Appendix P-II-D-1-a-(4). Personnel shall enter and exit the greenhouse facility only through the clothing change and shower rooms and shall shower each time they exit the greenhouse facility. Personnel shall use the airlocks to enter or exit the laboratory only in an emergency. In the event of an emergency, every reasonable effort should be made to prevent the possible transport of viable propagules from containment.

Appendix P-II-D-1-a-(5). Prior to entering the greenhouse, personnel shall be required to read and follow instructions on BL4-P practices and procedures.

Appendix P-II-D-1-b. Records (BL4-P)

Appendix P-II-D-1-b-(1). A record shall be kept of all experimental materials brought into or removed from the greenhouse.

Appendix P-II-D-1-b-(2). A record shall be kept of experiments currently in progress in the greenhouse facility.

Appendix P-II-D-1-b-(3). A record shall be kept of all personnel entering and exiting the greenhouse facility, including the date and time of each entry.

Appendix P-II-D-1-b-(4). The Principal Investigator shall report any greenhouse accident involving the inadvertent release or spill of microorganisms to the Biological Safety Officer, Greenhouse Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities immediately (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Documentation of any such accident shall be prepared and maintained.

Appendix P-II-D-1-c. Decontamination and Inactivation (BL4-P)

Appendix P-II-D-1-c-(1). All materials, except for those that are to remain in a viable or intact state for experimental purposes, shall be autoclaved prior to removal from the maximum containment greenhouse. Equipment or material that could be damaged by high temperatures or steam shall be decontaminated by alternative methods (e.g., gas or vapor sterilization) in an airlock or chamber designed for this purpose.

Appendix P-II-D-1-c-(2). Water that comes in contact with experimental microorganisms or with material exposed to such microorganisms (e.g., run-off from watering plants) shall be collected and decontaminated before disposal.

Appendix P-II-D-1-c-(3). Standard microbiological procedures shall be followed for decontamination of equipment and materials. Spray or liquid waste or rinse water from containers used to apply the experimental microorganisms shall be decontaminated before disposal.

Appendix P-II-D-1-d. Control of Undesired Species and Motile Macroorganisms (BL4-P)

Appendix P-II-D-1-d-(1). A chemical control program shall be implemented to eliminate undesired pests and pathogens in accordance with applicable state and Federal laws.

Appendix P-II-D-1-d-(2). Arthropods and other motile macroorganisms used in conjunction with experiments requiring BL4-P level physical containment shall be housed in appropriate cages. When appropriate to the organism, experiments shall be conducted within cages designed to contain the motile organisms.

Appendix P-II-D-1-e. Concurrent Experiments Conducted in the Greenhouse (BL4-P)

Appendix P-II-D-1-e-(1). Experiments involving organisms that require a containment level lower than BL4-P may be conducted in the greenhouse concurrently with experiments that require BL4-P containment provided that all work is conducted in accordance with BL4-P greenhouse practices. When the experimental microorganisms in use require a containment level lower than BL4-P, greenhouse practices reflect the level of containment required by the highest containment level microorganisms being tested.

Appendix P-II-D-1-f. Signs (BL4-P)

Appendix P-II-D-1-f-(1). A sign shall be posted indicating that a restricted experiment is in progress. The sign shall indicate the following: (i) the name of the responsible individual, (ii) the plants in use, and (iii) any special requirements for using the area.

Appendix P-II-D-1-f-(2). If organisms are used that have a recognized potential for causing serious detrimental impacts on managed or natural ecosystems, their presence shall be indicated by a sign posted on the greenhouse access doors.

Appendix P-II-D-1-f-(3). If there is a risk to human health, a sign shall be posted incorporating the universal biosafety symbol.

Appendix P-II-D-1-g. Transfer of Materials (BL4-P)

Appendix P-II-D-1-g-(1). Experimental materials that are brought into or removed from the greenhouse in a viable or intact state shall be transferred to a non-breakable, sealed, primary container then enclosed in a non-breakable, sealed secondary container. These containers shall be removed from the greenhouse facility through a chemical disinfectant, fumigation chamber, or an airlock designed for this purpose.

Appendix P-II-D-g-(2). Supplies and materials shall be brought into the greenhouse facility through a double-door autoclave, fumigation chamber, or airlock that is appropriately decontaminated between each use. After securing the outer doors, personnel within the greenhouse facility shall retrieve the materials by opening the interior door of the autoclave, fumigation chamber, or airlock. These doors shall be secured after the materials are brought into the greenhouse facility.

Appendix P-II-D-1-h. Greenhouse Practices Manual (BL4-P)

Appendix P-II-D-1-h-(1). A greenhouse practices manual shall be prepared or adopted. This manual shall include contingency plans to be implemented in the event of the unintentional release of experimental organisms.
Appendix P-II-D-1-i. Protective Clothing (BL4-P)

Appendix P-II-D-1-i-(1). Street clothing shall be removed in the outer clothing change room. Complete laboratory clothing (may be disposable) including undergarments, pants, and shirts, jump suits, shoes, and hats shall be provided and worn by all personnel entering the greenhouse facility.

Appendix P-II-D-1-i-(2). Personnel shall remove laboratory clothing when exiting the greenhouse facility and before entering the shower area. This clothing shall be stored in a locker or hamper in the inner change room.

Appendix P-II-D-1-i-(3). All laboratory clothing shall be autoclaved before laundering.

Appendix P-II-D-2. Facilities (BL4-P)

Appendix P-II-D-2-a. Greenhouse Design (BL4-P)

Appendix P-II-D-2-a-(1). The maximum containment greenhouse facility shall consist of a separate building or a clearly demarcated and isolated area within a building. The need to maintain negative pressure should be considered when constructing or renovating the greenhouse facility.

Appendix P-II-D-2-a-(2). Outer and inner change rooms, separated by a shower, shall be provided for personnel entering and exiting the greenhouse facility.

Appendix P-II-D-2-a-(3). Windows shall be closed and sealed. All glazing shall be resistant to breakage (e.g., double-pane tempered glass or equivalent).

Appendix P-II-D-2-a-(4). Access doors to the greenhouse shall be self-closing and locking.

Appendix P-II-D-2-a-(5). The greenhouse facility shall be surrounded by a security fence or protected by equivalent security measures.

Appendix P-II-D-2-a-(6). The walls, floors, and ceilings of the greenhouse shall be constructed to form a sealed internal shell that facilitates fumigation and is animal and arthropod-proof. These internal surfaces shall be resistant to penetration and degradation by liquids and chemicals to facilitate cleaning and decontamination of the area. All penetrations into these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix P-II-D-2-a-(7). Bench tops and other work surfaces shall have seamless surfaces impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix P-II-D-2-a-(8). A double-door autoclave, fumigation chamber, or ventilated airlock shall be provided for passage of all materials, supplies, or equipment that are not brought into the greenhouse facility through the change room.

Appendix P-II-D-2-b. Autoclaves (BL4-P)

Appendix P-II-D-2-b-(1). A double-door autoclave shall be provided for the decontamination of materials removed from the greenhouse facility. The autoclave door, which opens to the area external to the greenhouse facility, shall be sealed to the outer wall and automatically controlled so that it can only be opened upon completion of the sterilization cycle.

Appendix P-II-D-2-c. Supply and Exhaust Air Ventilation Systems (BL4-P)

Appendix P-II-D-2-c-(1). An individual supply and exhaust air ventilation system shall be provided. The system shall maintain pressure differentials and directional airflow as required to assure inward (or zero) airflow from areas outside of the greenhouse. Differential pressure transducers shall be used to sense pressure levels. If a system malfunctions, the transducers shall sound an alarm. A backup source of power should be considered. The supply and exhaust airflow shall be interlocked to assure inward (or zero) airflow at all times. The integrity of the greenhouse shall have an air leak rate (decay rate) not to exceed 7 percent per minute (logarithm of pressure against time) over a 20-minute period at 2 inches of water gauge pressure. Nominally, this is 0.05 inches of water gauge pressure loss in 1 minute at 2 inches water gauge pressure.

Appendix P-II-D-2-c-(2). Exhaust air from the greenhouse facility shall be filtered through high efficiency particulate air/HEPA filters and discharged to the outside and dispersed away from occupied buildings and air intakes. Filter chambers shall be designed to allow in situ decontamination before filters are removed and to facilitate certification testing after they are replaced. HEPA filters shall be provided to treat air supplied to the greenhouse facility. HEPA filters shall be certified annually.

Appendix P-II-D-2-d. Other (BL4-P)

Appendix P-II-D-2-d-(1). Sewer vents and other ventilation lines contain high efficiency particulate air/HEPA filters. HEPA filters shall be certified annually.

Appendix P-II-D-2-d-(2). A pass-through dunk tank, fumigation chamber, or an equivalent method of decontamination shall be provided to ensure decontamination of materials and equipment that cannot be decontaminated in the autoclave.

Appendix P-II-D-2-d-(3). Liquid effluent from sinks, floors, and autoclave chambers shall be decontaminated by heat or chemical treatment before being released from the maximum containment greenhouse facility. Liquid wastes from shower rooms and toilets may be decontaminated by heat or chemical treatment. Autoclave and chemical decontamination of liquid wastes shall be evaluated by appropriate standard procedures for autoclaved wastes. Decontamination shall be evaluated mechanically and biologically using a recording thermometer and an indicator microorganism with a defined heat susceptibility pattern. If liquid wastes are decontaminated with chemical disinfectants, the chemicals used must have demonstrated efficacy against the target or indicator microorganisms.

Appendix P-II-D-2-d-(4). If there is a central vacuum system, it shall not serve areas outside of the greenhouse facility. In-line high efficiency particulate air/HEPA filters shall be placed as near as practicable to each use point or vacuum service cock. Other liquid and gas services to the greenhouse facility shall be protected by devices that prevent back-flow. HEPA filters shall be certified annually.

Appendix P-III. Biological Containment Practices

Appropriate selection of the following biological containment practices may be used to meet the containment requirements for a given organism. The present list is not exhaustive; there may be other ways of preventing effective dissemination that could possibly lead to the establishment of the organism or its genetic material in the environment resulting in deleterious consequences to managed or natural ecosystems.

Appendix P-III-A. Biological Containment Practices (Plants)

Appendix P-III-A-1. Effective dissemination of plants by pollen or seed can be prevented by one or more of the following procedures: (i) cover the reproductive structures to prevent pollen dissemination at flowering and seed dissemination at maturity; (ii) remove reproductive structures by employing male sterile strains, or harvest the plant material prior to the reproductive stage; (iii) ensure that experimental plants flower at a time of year when cross-fertile plants are not flowering within the normal pollen dispersal range of the experimental plant; or (iv) ensure that cross-fertile plants are not growing within the known pollen dispersal range of the experimental plant.

Appendix P-III-B. Biological Containment Practices (Microorganisms)

Appendix P-III-B-1. Effective dissemination of microorganisms beyond the confines of the greenhouse can be prevented by one or more of the following procedures: (i) confine all operations to injections of microorganisms or other biological procedures (including genetic manipulation) that limit replication or reproduction of viruses and microorganisms or sequences derived from microorganisms, and confine these injections to internal plant parts or adherent plant surfaces; (ii) ensure that organisms, which can serve as hosts or promote the transmission of the virus or microorganism, are not present within the farthest distance that the airborne virus or microorganism may be expected to be effectively disseminated; (iii) conduct experiments at a time of year when plants that can serve as hosts are either not growing or are not susceptible to productive infection; (iv) use viruses and other microorganisms or their genomes that have known arthropod or animal vectors, in the absence of such vectors; (v) use microorganisms that have an obligate association with the plant; or (vi) use microorganisms that are genetically disabled to minimize survival outside of the research facility and whose natural mode of transmission requires injury of the target organism, or assures that inadvertent release is unlikely to initiate productive infection of organisms outside of the experimental facility.

Appendix P-III-C. Biological Containment Practices (Macroorganisms)

Appendix P-III-C-1. Effective dissemination of arthropods and other small animals can be prevented by using one or more of the following procedures: (i) use non-flying, flight-impaired, or sterile arthropods; (ii) use non-motile or sterile strains of small animals; (iii) conduct experiments at a time of year that precludes the survival of escaping organisms; (iv) use animals that have an obligate association with a plant that is not present within the dispersal range of the organism; or (v) prevent the escape of organisms present in run-off water by chemical treatment or evaporation of run-off water.

APPENDIX Q. PHYSICAL AND BIOLOGICAL CONTAINMENT FOR RECOMBINANT DNA RESEARCH INVOLVING ANIMALS

Appendix Q specifies containment and confinement practices for research involving whole animals, both those in which the animal's genome has been altered by stable introduction of recombinant DNA, or DNA derived therefrom, into the germ-line (transgenic animals) and experiments involving viable recombinant DNA-modified microorganisms tested on whole animals. The appendix applies to animal research activities with the following modifications:

Appendix Q shall supersede Appendix G when research animals are of a size or have growth requirements that preclude the use of containment for laboratory animals. Some animals may require other types of containment (see Appendix Q-III-D). The animals covered in Appendix Q are those species normally categorized as animals including but not limited to cattle, swine, sheep, goats, horses, and poultry.

The Institutional Biosafety Committee shall include at least one scientist with expertise in animal containment principles when experiments utilizing Appendix Q require Institutional Biosafety Committee prior approval.

The institution shall establish and maintain a health surveillance program for personnel engaged in animal research involving viable recombinant DNA-containing microorganisms that require Biosafety Level (BL) 3 or greater containment in the laboratory.

Appendix Q-I. General Considerations

Appendix Q-I-A. Containment Levels

The containment levels required for research involving recombinant DNA associated with or in animals is based on classification of experiments in Section III. For the purpose of animal research, four levels of containment are established. These are referred to as BL1-Animals (N), BL2-N, BL3-N, and BL4-N and are described in the following sections of Appendix Q. The descriptions include: (i) standard practices for physical and biological containment, and (ii) animal facilities.

Appendix Q-I-B. Disposal of Animals (BL1-N through BL4-N)

Appendix Q-I-B-1. When an animal covered by Appendix Q containing recombinant DNA or a recombinant DNA-derived organism is euthanized or dies, the carcass shall be disposed of to avoid its use as food for human beings or animals unless food use is specifically authorized by an appropriate Federal agency.

Appendix Q-I-B-2. A permanent record shall be maintained of the experimental use and disposal of each animal or group of animals.

Appendix Q-II. Physical and Biological Containment Levels

Appendix Q-II-A. Biosafety Level 1 - Animals (BL1-N)

Appendix Q-II-A-1. Standard Practices (BL1-N)

Appendix Q-II-A-1-a. Animal Facility Access (BL1-N)

Appendix Q-II-A-1-a-(1). The containment area shall be locked.

Appendix Q-II-A-1-a-(2). Access to the containment area shall be limited or restricted when experimental animals are being held.

Appendix Q-II-A-1-a-(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-A-1-b. Other (BL1-N)

Appendix Q-II-A-1-b-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-A-1-b-(2) A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-A-1-b-(3). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-A-2. Animal Facilities (BL1-N)

Appendix Q-II-A-2-(a). Animals shall be confined to securely fenced areas or be in enclosed structures (animal rooms) to minimize the possibility of theft or unintentional release.

Appendix Q-II-B. Biosafety Level 2 - Animals (BL2-N) (see Appendix Q-III-A)

Appendix Q-II-B-1. Standard Practices (BL2-N)

Appendix Q-II-B-1-a. Animal Facility Access (BL2-N)

Appendix Q-II-B-1-a-(1). The containment area shall be locked.

Appendix Q-II-B-1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-B-1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-B-1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal rooms.

Appendix Q-II-B-1-a-(5). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-B-1-b. Decontamination and Inactivation (BL2-N)

Appendix Q-II-B-1-b-(1). Contaminated materials that are decontaminated at a site away from the laboratory shall be placed in a closed durable leak-proof container prior to removal from the laboratory.

Appendix Q-II-B-1-b-(2). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B-1-c. Signs (BL2-N)

Appendix Q-II-B-1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-B-1-d. Protective Clothing (BL2-N)

Appendix Q-II-B-1-d-(1). Laboratory coats, gowns, smocks, or uniforms shall be worn while in the animal area or attached laboratory. Before entering non-laboratory areas (e.g., cafeteria, library, administrative offices), protective clothing shall be removed and kept in the work entrance area.

Appendix Q-II-B-1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-B-1-e. Records (BL2-N)

Appendix Q-II-B-1-e-(1). Any incident involving spills and accidents that result in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-B-1-e-(2). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled and the function of the animal facility.

Appendix Q-II-B-1-f. Transfer of Materials (BL2-N)

Appendix Q-II-B-1-f-(1). Biological materials removed from the animal containment area in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may only be opened in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-B-1-g. Other (BL2-N)

Appendix Q-II-B-1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-B-1-g-(2). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-B-1-g-(3). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as a vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste, etc.) should be prevented.

Appendix Q-II-B-1-g-(4). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-B-1-g-(5). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-B-1-g-(6). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-B-1-g-(7). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-B-1-g-(8). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-B-2. Animal Facilities (BL2-N)

Appendix Q-II-B-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and to avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-B-2-b. Surfaces shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

Appendix Q-II-B-2-c. The animal containment area shall be designed so that it can be easily cleaned.

Appendix Q-II-B-2-d. Windows that open shall be fitted with fly screens.

Appendix Q-II-B-2-e. An autoclave shall be available for decontamination of laboratory wastes.

Appendix Q-II-B-2-f. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, interior work areas shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening of access doors or the equivalent.

Appendix Q-II-C. Biosafety Level 3 - Animals (BL3-N) (see Appendix Q-III-B)

Appendix Q-II-C-1. Standard Practices (BL3-N)

Appendix Q-II-C-1-a. Animal Facility Access (BL3-N)

Appendix Q-II-C-1-a-(1). The containment area shall be locked.

Appendix Q-II-C-1-a-(2). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-C-1-a-(3). The containment building shall be controlled and have a locking access.

Appendix Q-II-C-1-a-(4). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) shall enter the laboratory or animal rooms.

Appendix Q-II-C-1-a-(5). Animal room doors, gates, or other closures shall be kept closed when experiments are in progress.

Appendix Q-II-C-1-b. Decontamination and Inactivation (BL3-N)

Appendix Q-II-C-1-b-(1). The work surfaces of containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q-II-C-1-b-(2). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C-1-b-(3). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C-1-b-(4). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

Appendix Q-II-C-1-b-(5). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C-1-c. Signs (BL3-N)

Appendix Q-II-C-1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-C-1-d. Protective Clothing (BL3-N)

Appendix Q-II-C-1-d-(1). Full protective clothing that protects the individual (e.g., scrub suits, coveralls, uniforms) shall be worn in the animal area. Clothing shall not be worn outside the animal containment area and

shall be decontaminated before laundering or disposal. Personnel shall be required to shower before exiting the BL3-N area and wearing of personal clothing.

Appendix Q-II-C-1-d-(2). Special care shall be taken to avoid skin contamination with microorganisms containing recombinant DNA. Impervious and/or protective gloves shall be worn when handling experimental animals and when skin contact with an infectious agent is unavoidable.

Appendix Q-II-C-1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-C-1-e. Records (BL3-N)

Appendix Q-II-C-1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-C-1-e-(2). Any incident involving spills and accidents that result in environmental release or exposure of animals or laboratory workers to organisms containing recombinant DNA shall be reported immediately to the Biological Safety Office, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland 20892, (301) 496-9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-C-1-e-(3). When appropriate and giving consideration to the agent handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agent handled or the function of the facility.

Appendix Q-II-C-1-f. Transfer of Materials (BL3-N)

Appendix Q-II-C-1-f-(1). Biological materials removed from the animal containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container. All containers, primary and secondary, shall be disinfected before removal from the animal facility. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Packages containing viable agents may be opened only in a facility having an equivalent or higher level of physical containment unless the agent is biologically inactivated or incapable of reproduction.

Appendix Q-II-C-1-f-(2). Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL3-N animal facility to a facility with a lower containment classification.

Appendix Q-II-C-1-g. Other (BL3-N)

Appendix Q-II-C-1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-C-1-g-(2). Appropriate steps should be taken to prevent horizontal transmission or exposure of laboratory personnel. If the agent used as the vector is known to be transmitted by a particular route (e.g., arthropods), special attention should be given to preventing spread by that route. In the absence of specific knowledge of a particular route of transmission, all potential means of horizontal transmission (e.g., arthropods, contaminated bedding, or animal waste) should be prevented.

Appendix Q-II-C-1-g-(3). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-C-1-g-(4). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-C-1-g-(5). Experiments involving other organisms that require containment levels lower than BL3-N may be conducted in the same area concurrently with experiments requiring BL3-N containment provided that they are conducted in accordance with BL3-N practices.

Appendix Q-II-C-1-g-(6). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-C-1-g-(7). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-C-1-g-(8). A double barrier shall be provided to separate male and female animals unless reproductive studies are part of the experiment or other measures are taken to avoid reproductive transmission. Reproductive incapacitation may be used.

Appendix Q-II-C-1-g-(9). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-C-1-g-(10). All animals shall be euthanized at the end of their experimental usefulness and the carcasses decontaminated before disposal in an approved manner.

Appendix Q-II-C-1-g-(11). Personnel shall be required to shower before exiting the BL3-N area and wearing personal clothing.

Appendix Q-II-C-1-g-(12). Animals of the same or different species, which are not involved in the work being performed, shall not be permitted in the animal area.

Appendix Q-II-C-1-g-(13). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-C-1-g-(14). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-C-2. Animal Facilities (BL3-N)

Appendix Q-II-C-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access. The special provision to avoid the entry or escape of arthropods from the animal areas may be waived if the agent in use is not known to be transmitted by arthropods.

Appendix Q-II-C-2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-C-2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent). The need to maintain negative pressure should be considered when constructing or renovating the animal facility.

Appendix Q-II-C-2-d. An autoclave, incinerator, or other effective means to decontaminate animals and waste shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-C-2-e. If arthropods are used in the experiment or the agent under study can be transmitted by an arthropod, the interior work area shall be appropriately screened (52 mesh). All perimeter joints and openings shall be sealed, and additional arthropod control mechanisms used to minimize arthropod entry and propagation, including appropriate screening, or the equivalent of access doors.

Appendix Q-II-C-2-f. Access doors to the containment area shall be self-closing.

Appendix Q-II-C-2-g. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. The animal containment area shall be physically separated from access corridors and other laboratories or areas by a double-door clothes change room, equipped with integral showers and airlock.

Appendix Q-II-C-2-h. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism.

Appendix Q-II-C-2-i. An exhaust air ventilation system shall be provided. This system shall create directional airflow that draws air into the animal room through the entry area. The building exhaust, or the exhaust from primary containment units, may be used for this purpose if the exhaust air is discharged to the outside and shall be dispersed away from occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-C-2-j. If the agent is transmitted by aerosol, then the exhaust air shall pass through a high efficiency particulate air/HEPA filter.

Appendix Q-II-C-2-k. Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-C-2-l. In lieu of open housing in the special animal room, animals held in a BL3-N area may be housed in partial-containment caging systems (e.g., Horsfall units or gnotobiotic systems, or other special containment primary barriers). Prudent judgment must be exercised to implement this ventilation system (e.g., animal species) and its discharge location.

Appendix Q-II-C-2-m. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q-II-C-2-n. Restraining devices for animals may be required to avoid damage to the integrity of the animal containment facility.

Appendix Q-II-D. Biosafety Level 4 - Animals (BL4-N) (see Appendix Q-III-C)

Appendix Q-II-D-1. Standard Practices (BL4-N)

Appendix Q-II-D-1-a. Animal Facility Access (BL4-N)

Appendix Q-II-D-1-a-(1). Individuals under 16 years of age shall not be permitted to enter the animal area.

Appendix Q-II-D-1-a-(2). The containment area shall be locked.

Appendix Q-II-D-1-a-(3). The containment area shall be patrolled or monitored at frequent intervals.

Appendix Q-II-D-1-a-(4). The containment building shall be controlled and have a locking access.

Appendix Q-II-D-1-a-(5). The Animal Facility Director shall establish policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) may enter the laboratory or animal room.

Appendix Q-II-D-1-a-(6). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms.

Appendix Q-II-D-1-a-(7). Personnel shall use the airlocks to enter or exit the laboratory only in an emergency.

Appendix Q-II-D-1-a-(8). Animal room doors, gates, and other closures shall be kept closed when experiments are in progress.

Appendix Q-II-D-1-b. Decontamination and Inactivation (BL4-N)

Appendix Q-II-D-1-b-(1). All contaminated liquid or solid wastes shall be decontaminated before disposal.

Appendix Q-II-D-1-b-(2). The work surfaces and containment equipment shall be decontaminated when work with organisms containing recombinant DNA molecules is finished. Where feasible, plastic-backed paper toweling shall be used on nonporous work surfaces to facilitate clean-up.

Appendix Q-II-D-1-b-(3). All wastes from animal rooms and laboratories shall be appropriately decontaminated before disposal in an approved manner.

Appendix Q-II-D-1-b-(4). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D-1-b-(5). When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D-1-b-(6). Needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D-1-b-(7). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use.

Appendix Q-II-D-1-b-(8). An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D-1-b-(9). Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D-1-c. Signs (BL4-N)

Appendix Q-II-D-1-c-(1). When the animal research requires special provisions for entry (e.g., vaccination), a warning sign incorporating the universal biosafety symbol shall be posted on all access doors to the animal work area. The sign shall indicate: (i) the agent, (ii) the animal species, (iii) the name and telephone number of the Animal Facility Director, or other responsible individual, and (iv) any special requirements for entering the laboratory.

Appendix Q-II-D-1-d. Protective Clothing (BL4-N)

Appendix Q-II-D-1-d-(1). Individuals shall enter and exit the animal facility only through the clothing change and shower rooms. Street clothing shall be removed and kept in the outer clothing change room. Complete laboratory clothing (may be disposable), including undergarments, pants, shirts, jump suits, and shoes shall be provided for all personnel entering the animal facility. When exiting the BL4-N area and before proceeding into the shower area, personnel shall remove their laboratory clothing in the inner change room. All laboratory clothing shall be autoclaved before laundering. Personnel shall shower each time they exit the animal facility.

Appendix Q-II-D-1-d-(2). A ventilated head-hood or a one-piece positive pressure suit, which is ventilated by a life-support system, shall be worn by all personnel entering rooms that contain experimental animals when appropriate. When ventilated suits are required, the animal personnel shower entrance/exit area shall be equipped with a chemical disinfectant shower to decontaminate the surface of the suit before exiting the area. A neutralization or water dilution device shall be integral with the chemical disinfectant discharge piping before entering the heat sterilization system. Entry to this area shall be through an airlock fitted with airtight doors.

Appendix Q-II-D-1-d-(3). Appropriate respiratory protection shall be worn in rooms containing experimental animals.

Appendix Q-II-D-1-e. Records (BL4-N)

Appendix Q-II-D-1-e-(1). Documents regarding experimental animal use and disposal shall be maintained in a permanent record book.

Appendix Q-II-D-1-e-(2). A system shall be established for: (i) reporting laboratory accidents and exposures that are a result of overt exposures to organisms containing recombinant DNA, (ii) employee absenteeism, and (iii) medical surveillance of potential laboratory-associated illnesses. Permanent records shall be prepared and maintained. Any incident involving spills and accidents that results in environmental release or exposures of animals or laboratory workers to organisms containing recombinant DNA molecules shall be reported immediately to the Biological Safety Officer, Animal Facility Director, Institutional Biosafety Committee, NIH/ORDA, and other appropriate authorities (if applicable). Reports to the NIH/ORDA shall be sent to the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 4B11, Bethesda, Maryland

20892, (301) 496-9838. Medical evaluation, surveillance, and treatment shall be provided as appropriate and written records maintained. If necessary, the area shall be appropriately decontaminated.

Appendix Q-II-D-1-e-(3). When appropriate and giving consideration to the agents handled, baseline serum samples shall be collected and stored for animal care and other at-risk personnel. Additional serum specimens may be collected periodically depending on the agents handled or the function of the facility.

Appendix Q-II-D-1-e-(4). A permanent record book indicating the date and time of each entry and exit shall be signed by all personnel.

Appendix Q-II-D-1-f. Transfer of Materials (BL4-N)

Appendix Q-II-D-1-f-(1). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the maximum containment laboratory unless they have been autoclaved or decontaminated. Equipment or material that might be damaged by high temperatures or steam shall be decontaminated by gaseous or vapor methods in an airlock or chamber designed for this purpose.

Appendix Q-II-D-1-f-(2). Biological materials removed from the animal maximum containment laboratory in a viable or intact state shall be transferred to a non-breakable sealed primary container and then enclosed in a non-breakable sealed secondary container that shall be removed from the animal facility through a disinfectant dunk tank, fumigation chamber, or an airlock designed for this purpose. Advance approval for transfer of material shall be obtained from the Animal Facility Director. Such packages containing viable agents can only be opened in another BL4-N animal facility if the agent is biologically inactivated or incapable of reproduction. Special safety testing, decontamination procedures, and Institutional Biosafety Committee approval shall be required to transfer agents or tissue/organ specimens from a BL4-N animal facility to one with a lower containment classification.

Appendix Q-II-D-1-f-(3). Supplies and materials needed in the animal facility shall be brought in by way of the double-door autoclave, fumigation chamber, or airlock that shall be appropriately decontaminated between each use. After securing the outer doors, personnel within the animal facility retrieve the materials by opening the interior doors of the autoclave, fumigation chamber, or airlock. These doors shall be secured after materials are brought into the animal facility.

Appendix Q-II-D-1-g. Other (BL4-N)

Appendix Q-II-D-1-g-(1). All genetically engineered neonates shall be permanently marked within 72 hours after birth, if their size permits. If their size does not permit marking, their containers should be marked. In addition, transgenic animals should contain distinct and biochemically assayable DNA sequences that allow identification of transgenic animals from among non-transgenic animals.

Appendix Q-II-D-1-g-(2). Eating, drinking, smoking, and applying cosmetics shall not be permitted in the work area.

Appendix Q-II-D-1-g-(3). Individuals who handle materials and animals containing recombinant DNA molecules shall be required to wash their hands before exiting the containment area.

Appendix Q-II-D-1-g-(4). Experiments involving other organisms that require containment levels lower than BL4-N may be conducted in the same area concurrently with experiments requiring BL4-N containment provided that they are conducted in accordance with BL4-N practices.

Appendix Q-II-D-1-g-(5). Animal holding areas shall be cleaned at least once a day and decontaminated immediately following any spill of viable materials.

Appendix Q-II-D-1-g-(6). All procedures shall be performed carefully to minimize the creation of aerosols.

Appendix Q-II-D-1-g-(7). A double barrier shall be provided to separate male and female animals. Animal isolation barriers shall be sturdy and accessible for cleaning. Reproductive incapacitation may be used.

Appendix Q-II-D-1-g-(8). The containment area shall be in accordance with state and Federal laws and animal care requirements.

Appendix Q-II-D-1-g-(9). The life support system for the ventilated suit or head hood is equipped with alarms and emergency back-up air tanks. The exhaust air from the suit area shall be filtered by two sets of high efficiency particulate air/HEPA filters installed in series or incinerated. A duplicate filtration unit, exhaust fan, and an automatically starting emergency power source shall be provided. The air pressure within the suit shall be greater than that of any adjacent area. Emergency lighting and communication systems shall be provided. A double-door autoclave shall be provided for decontamination of waste materials to be removed from the suit area.

Appendix Q-II-D-1-g-(10). Needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) shall be used for the injection or aspiration of fluids containing organisms that contain recombinant DNA. Extreme caution shall be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Following use, needles shall not be bent, sheared, replaced in the needle sheath or guard, or removed from the syringe. The needles and syringes shall be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.

Appendix Q-II-D-1-g-(11). An essential adjunct to the reporting-surveillance system is the availability of a facility for quarantine, isolation, and medical care of personnel with potential or known laboratory-associated illnesses.

Appendix Q-II-D-1-g-(12). A biosafety manual shall be prepared or adopted. Personnel shall be advised of special hazards and required to read and follow instructions on practices and procedures.

Appendix Q-II-D-1-g-(13). Vacuum lines shall be protected with high efficiency particulate air/HEPA filters and liquid disinfectant traps.

Appendix Q-II-D-2. Animal Facilities (BL4-N)

Appendix Q-II-D-2-a. Animals shall be contained within an enclosed structure (animal room or equivalent) to minimize the possibility of theft or unintentional release and avoid arthropod access.

Appendix Q-II-D-2-b. The interior walls, floors, and ceilings shall be impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat, to facilitate cleaning. Penetrations in these structures and surfaces (e.g., plumbing and utilities) shall be sealed.

Appendix Q-II-D-2-c. Windows in the animal facility shall be closed, sealed, and breakage resistant (e.g., double-pane tempered glass or equivalent).

Appendix Q-II-D-2-d. An autoclave, incinerator, or other effective means to decontaminate animals and wastes shall be available, preferably within the containment area. If feasible, a double-door autoclave is preferred and should be positioned to allow removal of material from the containment area.

Appendix Q-II-D-2-e. Access doors to the containment area shall be self-closing.

Appendix Q-II-D-2-f. All perimeter joints and openings shall be sealed to form an arthropod-proof structure.

Appendix Q-II-D-2-g. The BL4-N laboratory provides a double barrier to prevent the release of recombinant DNA containing microorganisms into the environment. Design of the animal facility shall be such that if the barrier of the inner facility is breached, the outer barrier will prevent release into the environment. The animal area shall be separated from all other areas. Passage through two sets of doors shall be the basic requirement for entry into the animal area from access corridors or other contiguous areas. Physical separation of the animal containment area from access corridors or other laboratories or activities shall be provided by a double-door clothes change room equipped with integral showers and airlock.

Appendix Q-II-D-2-h. A necropsy room shall be provided within the BL4-N containment area.

Appendix Q-II-D-2-i. Liquid effluent from containment equipment, sinks, biological safety cabinets, animal rooms, primary barriers, floor drains, and sterilizers shall be decontaminated by heat treatment before being released into the sanitary system. Liquid wastes from shower rooms and toilets shall be decontaminated with chemical disinfectants or heat by methods demonstrated to be effective. The procedure used for heat decontamination of liquid wastes shall be monitored with a recording thermometer. The effectiveness of the heat decontamination process system shall be revalidated every 30 days with an indicator organism. Liquid wastes from the shower shall be chemically decontaminated using an Environmental Protection Agency-approved germicide. The efficacy of the chemical treatment process shall be validated with an indicator organism. Chemical disinfectants shall be neutralized or diluted before release into general effluent waste systems.

Appendix Q-II-D-2-j. A ducted exhaust air ventilation system shall be provided that creates directional airflow that draws air into the laboratory through the entry area. The exhaust air, which is not recirculated to any other area of the building, shall be discharged to the outside and dispersed away from the occupied areas and air intakes. Personnel shall verify that the direction of the airflow (into the animal room) is proper.

Appendix Q-II-D-2-k. Exhaust air from BL4-N containment area shall be double high efficiency particulate air/HEPA filtered or treated by passing through a certified HEPA filter and an air incinerator before release to the atmosphere. Double HEPA filters shall be required for the supply air system in a BL4-N containment area.

Appendix Q-II-D-2-l. All high efficiency particulate air/HEPA filters' frames and housings shall be certified to have no detectable smoke [dioctylphthalate] leaks when the exit face (direction of flow) of the filter is scanned above 0.01 percent when measured by a linear or logarithmic photometer. The instrument must demonstrate a threshold sensitivity of at least 1x10-3 micrograms per liter for 0.3 micrometer diameter dioctylphthalate particles and a challenge concentration of 80-120 micrograms per liter. The air sampling rate should be at least 1 cfm (28.3 liters per minute).

Appendix Q-II-D-2-m. If an air incinerator is used in lieu of the second high efficiency particulate air/HEPA filter, it shall be biologically challenged to prove all viable test agents are sterilized. The biological challenge must be minimally 1x108 organisms per cubic foot of airflow through the incinerator. It is universally accepted if bacterial spores are used to challenge and verify that the equipment is capable of killing spores, then assurance is provided that all other known agents are inactivated by the parameters established to operate the equipment. Test spores meeting this criterion are Bacillus subtilis var. niger or Bacillus stearothermophilis. The operating temperature of the incinerator shall be continuously monitored and recorded during use.

Appendix Q-II-D-2-n. All equipment and floor drains shall be equipped with deep traps (minimally 5 inches). Floor drains shall be fitted with isolation plugs or fitted with automatic water fill devices.

Appendix Q-II-D-2-o. Each animal area shall contain a foot, elbow, or automatically operated sink for hand washing. The sink shall be located near the exit door.

Appendix Q-II-D-2-p. Restraining devices for animals may be required to avoid damage to the integrity of the containment animal facility.

Appendix Q-II-D-2-q. The supply water distribution system shall be fitted with a back-flow preventer or break tank.

Appendix Q-II-D-2-r. All utilities, liquid and gas services, shall be protected with devices that avoid back-flow.

Appendix Q-II-D-2-s. Sewer and other atmospheric ventilation lines shall be equipped minimally with a single high efficiency particulate/HEPA filter. Condensate drains from these type housings shall be appropriately connected to a contaminated or sanitary drain system. The drain position in the housing dictates the appropriate system to be used.

Appendix Q-III. Footnotes and References for Appendix Q

Appendix Q-III-A. If recombinant DNA is derived from a Class 2 organism requiring BL2 containment, personnel shall be required to have specific training in handling pathogenic agents and directed by knowledgeable scientists.

Appendix Q-III-B. Personnel who handle pathogenic and potentially lethal agents shall be required to have specific training and be supervised by knowledgeable scientists who are experienced in working with these agents. BL3-N containment also minimizes escape of recombinant DNA-containing organisms from exhaust air or waste material from the containment area.

Appendix Q-III-C. Classes 4 and 5 microorganisms pose a high level of individual risk for acquiring life-threatening diseases to personnel and/or animals. To import Class 5 agents, special approval must be obtained from U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Import-Export Products, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

Laboratory staff shall be required to have specific and thorough training in handling extremely hazardous infectious agents, primary and secondary containment, standard and special practices, and laboratory design characteristics. The laboratory staff shall be supervised by knowledgeable scientists who are trained and experienced in working with these agents and in the special containment facilities.

Within work areas of the animal facility, all activities shall be confined to the specially equipped animal rooms or support areas. The maximum animal containment area and support areas shall have special engineering and design features to prevent the dissemination of microorganisms into the environment via exhaust air or waste disposal.

Appendix Q-III-D. Other research with non-laboratory animals, which may not appropriately be conducted under conditions described in Appendix Q, may be conducted safely by applying practices routinely used for controlled culture of these biota. In aquatic systems, for example, BL1 equivalent conditions could be met by utilizing growth tanks that provide adequate physical means to avoid the escape of the aquatic species, its gametes, and introduced exogenous genetic material. A mechanism shall be provided to ensure that neither the organisms nor their gametes can escape into the supply or discharge system of the rearing container (e.g., tank, aquarium, etc.) Acceptable barriers include appropriate filtration, irradiation, heat treatment, chemical treatment, etc. Moreover, the top of the rearing container shall be covered to avoid escape of the organism and its gametes. In the event of tank rupture, leakage, or overflow, the construction of the room containing these tanks should prevent the organisms and gametes from entering the building's drains before the organism and its gametes have been inactivated.

Other types of non-laboratory animals (e.g., nematodes, arthropods, and certain forms of smaller animals) may be accommodated by using the appropriate BL1 through BL4 or BL1-P through BL4-P containment practices and procedures as specified in Appendices G and P.

<u>Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592)</u>

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the Catalog of Federal Domestic Assistance. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected.

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Harold Varmus, M.D.
Director
National Institutes of Health

BIOHAZARD CONTAINMENT EQUIPMENT

A. TYPES OF CONTAINMENT EQUIPMENT

Control of microbiological containment in the laboratory can be achieved by proper use of containment equipment. It is generally accepted that such equipment is most effective if the potential hazard is enclosed at the source i.e., at the work site. Laboratory containment equipment can be described as either "partial barrier" or "absolute barrier". Partial barrier equipment depends upon negative air pressure and airflow directions to achieve containment. Absolute barrier equipment provides a separated negative pressure working environment when manipulations are done through arm-length rubber gloves. These types of containment equipment include, for example, the following:

PARTIAL BARRIER	*ABSOLUTE BARRIER*
Filter Top Animal Cage	Class III Gas-Tight Cabinet
Class I Open Face Safety Cabinet	Class III Cage Systems
Class II Biological Safety Cabinet	Germ Free Animal Chambers
	Gas-Tight Centrifuge Chamber
	Gas-Tight Blender Units

Each of these examples offers specific solutions to biological containment. Their utilization is dependent on the nature of the experiment and/or risk involved.

B. USE OF CONTAINMENT EQUIPMENT

1. A suitably ventilated biological safety cabinet is recommended for all procedures with biohazardous materials such as; opening test tubes, flasks and bottles, doing dissections, using pipettes, making dilutions, inoculating or autopsying animals, grinding tissue, blending cultures, opening lyophile tubes, and operating ultrasonic disintegrators.

2. A chemical fume hood may sometimes be an acceptable substitute for use with biological toxic substances. The supervisor on advice of the Biosafety Officer is responsible for insuring proper use of such devices.

3. Ventilated containment devices are also recommended for placement around shaking machines and centrifuges. Safety centrifuge cups should be used when available. When centrifuging is done in a Class III biological safety cabinet, the glove panel

should be in place because an operating centrifuge can create reverse air currents that might allow escape of the agent from an open cabinet.

4. Prior to use, all biological safety cabinets, laminar flow devices or any other ventilated containment devices shall be tested by qualified personnel to determine the adequacy of the equipment.

5. A respiratory protective device (NIOSH dust/mist respirator) should be worn when changing gloves attached to a safety cabinet if an infectious aerosol may possibly be present in the cabinet. For toxic or chemical agents, advice may be obtained from The Johns Hopkins Institutions Environmental Health Officer.

6. Frequent washdowns of biological safety cabinets are recommended to prevent buildup of infectious agents. In no instance should such a use of disinfectant solution be considered a sterilization procedure.

C. BIOHAZARD CABINET CONTAINMENT EQUIPMENT

Control of microbiological containment in the laboratory can be achieved by proper selection and use of ventilated cabinet containment equipment. It is generally accepted that potentially hazardous materials should be controlled at the source to protect personnel, product, and the environment.

The most elementary ventilated cabinet (sometimes incorrectly called a "tissue culture hood") can be described as a metal box approximately four to six feet in length, and approximately four feet high. These cabinets are secured to a stand or base cabinet. The cabinet accommodates a waist high work surface which is usually about three feet deep. The user sits at the open front of the cabinet and an electric blower either pulls air towards the rear of the cabinet or blows air toward the user. Some ventilated cabinets require a physical duct connection to an ancillary air handling system, i.e. building exhaust system or dedicated exhaust system.

The heart of a ventilated cabinet is the high efficiency particulate air (HEPA) filter. The HEPA filter is positioned within the cabinets' ducting. The HEPA filter removes particulate material, such as dust, fungal spores, bacteria, and viruses from the internal cabinet air. This HEPA-filtered air is then directed to either the laboratory (worker protection) or to the work surface (product protection).

Over the years, ventilated cabinet technology advanced to more than just "a box on a stand". Manufacturers have produced many sophisticated model designs with special safety protective functions. Some ventilated cabinets are designed to protect only

personnel, not the work product or environment. Other ventilated cabinets provide personnel and product protection but no protection for the environment. Hence, there are many variations of the ventilated cabinet design. Therefore, all ventilated cabinets are not the same, despite their external appearance. Prior to selecting a cabinet, you need to carefully evaluate the level of safety protection needed for each work application and special needs of the research.

A common error people make with ventilated cabinets is mistaking a chemical fume hood (hood) or clean air bench (CAB) for a specialized cabinet called a biological safety cabinet (BSC). Only annually certified BSC's should be used with biohazardous materials. The chemical fume hood, or "hood", is specifically designed for use with potentially hazardous chemicals and is not generally used with potentially biohazardous materials. The CAB is designed to provide a clean work area for assembling electronic components. In some cases a CAB may be approved by the Office of Safety and Environmental Health (OSEH) for sterile media preparation or some pharmaceutical preparations.

NEED MORE INFORMATION?

Since, ventilated cabinets have a striking external resemblance to one another, how do you determine what kind of ventilated cabinet is installed in your lab and whether it is safe and proper for your work? Ask the Principal Investigator(PI), Laboratory Director or Supervisor, read the rest of this manual, and call the OSEH Biosafety Officer at 955-5918 for assistance.

The Johns Hopkins Institutions Office of Safety and Environmental Health (OSEH) has a computer data base with files of all operating and stored ventilated cabinets within the Johns Hopkins Institutions. If you want to identify or receive technical information about a particular ventilated cabinet in a laboratory or what is new and available from industry, call the Biosafety Officer for assistance. OSEH will need the following cabinet information: manufacturer, model, serial number, building, laboratory room number, and certification date.

BIOLOGICAL SAFETY CABINET CATEGORIES

A. Biological safety cabinets (BSC's) are broken down into two major containment or barrier categories: "partial" and "absolute". These two categories are further broken down into Classes and Types:

PARTIAL BARRIER	**ABSOLUTE BARRIER**
Class I Open Face Safety Cabinet	Class III Gas-Tight Cabinet
Class II, Type A, B1, B2, B3 Biological Safety Cabinet	Class III Cage Systems

B. Partial barrier BSC's are open to the atmosphere on one side. They depend upon constant directional negative airflow to provide containment. High efficiency particulate air (HEPA) filters cleanse potentially contaminated exhaust air before it is exhausted into the external environment (laboratory). Containment of hazardous materials can be compromised if a partial barrier cabinets' inward airflow (blower motor) is shut off.

C. Absolute barrier BSC's are enclosed on all sides. They provide a separate directional negative air pressure working environment when manipulations are done through arm-length rubber gloves. Introduction or removal of materials through an absolute barrier BSC is managed through special dunk tanks and isolation chambers with gasketed doors. Air entering and leaving the absolute barrier BSC is HEPA filtered.

D. Both partial and absolute BSC's offer specific solutions to specific biohazard containment requirements. As you will see, each BSC "Class" and "Type" offer different operational performance characteristics which must be evaluated carefully. BSC characteristic needed for a particular project depends on the nature of the experiment and risk involved. Therefore, the purchase, selection, use, and "certification" (performance testing) of all BSC's must be coordinated through the Biosafety Officer.

E. Class I - Open Face Biological Safety Cabinets:

DESCRIPTION: A partial barrier biological safety (BSC) with unfiltered, non-recirculated inward airflow away from the operator and over the work surface.

USE: Class I BSC's provide worker and environmental protection only. Class I BSC's do not provide product protection. Class I's may be used for manipulations at Biosafety Level 1, 2 and 3 when no experiment (product) protection is required.

Note: To provide environmental protection, Class I BSC exhaust air must pass through a high efficiency particulate air (HEPA) filter before being discharged to the outside atmosphere.

F. Class II Biological Safety Cabinets:

Class II biological safety cabinets (BSC's) are broken down into four "Types" with specific performance characteristics. These Types are: Class II, Type A; Class II, Type B1; Class II, Type B2; and Class II, Type B3.

DESCRIPTION: The Class II BSC's are partial barrier ventilated cabinets. Incorrect technical slang words used for these BSC's are "hood" or "tissue culture hood". The correct technical name is "biological safety cabinet" or the acronym "BSC". The "Type" of BSC dictates the configuration of the cabinet. The BSC configuration determines what percentage of internal BSC air is recirculated within the BSC and what percentage of air is exhausted from the BSC.

Air flows into Class II BSC's through the front work access opening and is drawn away from the operator. This provides personnel protection. Class II BSC's have either one or two HEPA filters within the cabinet, depending on the cabinet Type. Class II, Type A, B1, and B3 BSC's have one supply air HEPA filter and one exhaust air HEPA filter. Class II, Type B2 BSC's typically have one internal supply air HEPA filter. The Class II, Type B2 exhaust air is typically HEPA filtered through an ancillary HEPA filter installed upstream in a dedicated building exhaust system.

The supply HEPA filter provides particle-free vertical laminar airflow over the work surface, which protects the product from contamination.

The exhaust HEPA filter provides particle-free BSC exhaust air. This prevents potentially biohazardous material used in the BSC from escaping into the environment (laboratory).

USE: Class II Biological Safety Cabinets (BSC's) provide personnel, product, and environmental protection. Class II BSC's must be used for all activities involving low or moderate risk biohazardous agents, etiologic agents, oncogenic viruses, and recombinant DNA experiments requiring Biosafety Level 1, 2, and 3 containment.

G. Class III Gas-Tight Biological Safety Cabinets:

DESCRIPTION: An absolute barrier BSC with HEPA filtered, non-recirculated airflow isolated from the operator via a physical barrier, such as a wall and rubber gloves. Horizontal and directional laminar airflow is provided over the work surface.

USE: This equipment provides the highest containment reliability and should be used for all activities involving high risk biohazards (such as high risk etiologic agents, high risk oncogenic agents, and recombinant DNA experiments requiring Biosafety Level 4 containment).

NOTE: Some laboratories use cabinets with restricted work openings and ultraviolet lights, but without any high efficiency particulate air (HEPA) filtered air exhaust capability. These cabinets are not classified as biological safety cabinets and are **not approved** for use with potentially infectious agents or materials. These cabinets do not provide worker or environment protection.

USE OF BIOLOGICAL SAFETY CABINETS

A. Know the potential biohazard of the etiologic agent or material to be used in the BSC.

B. Know the Class and Type of biological safety cabinet (BSC) you are about to use prior to using it. Be familiar with the weaknesses and strengths of the Class and Type of BSC selected. Occasionally, use of a chemical fume hood may be permitted. Call the Biosafety Officer for assistance and additional information.

C. Procedures to be carried out in BSC's include:

1. Opening test tubes, flasks, and bottles,
2. Dissections or necropsy,
3. Pipetting,
4. Making dilutions,
5. Injecting animals,
6. Grinding tissue,
7. Blending cultures,
8. Opening lyophile tubes, and
9. Operating ultrasonic disintegrators, vortex mixers or other high energy equipment.

D. The Principal Investigator or Laboratory Director, on advice of the Biosafety Officer, is responsible for ensuring proper personnel safety and use of ventilated cabinets in the lab. Refer to the Johns Hopkins Institutions Biohazards Safety Manual or call the Biosafety Officer.

E. At times there may be requirements for special cabinets to protect workers from airborne transmission of biohazardous agents or materials. Cabinets may be required for shaking machines, animal waste containers, and centrifuges.

F. Prior to use, check the status of your BSC. All laminar flow BSC's must be performance tested ("certified") annually by qualified personnel.

G. Employ special safety work practices designed for the work you are doing. If you are not sure what practices you need to follow, talk to your principal investigator or call the Biosafety Officer.

H. Disinfect all work surfaces before, during and after BSC use. Frequent disinfection of BSC surfaces is required by national and university biosafety guidelines.

OPERATION OF BIOLOGICAL SAFETY CABINETS

A. GENERAL SUGGESTIONS

1. Keep your laboratory meticulously clean. Minimize storage of boxes and supplies particularly near a BSC. Never place boxes on top of a BSC.

2. Wash your hands thoroughly before and after working in your BSC. Wearing a clean lab coat and gloves while working in a BSC increases your safety and helps reduce contamination of research materials.

3. The effectiveness of a BSC is a function of directional airflow, inward and downward, through a high efficiency particulate air filter (HEPA). Anything that disrupts the airflow pattern reduces cabinet effectiveness, such as; rapidly moving your arms in and out of the BSC, people walking behind you, down-drafts from ventilation systems and drafts from open laboratory doors.

4. Understand how your BSC works. Plan your work ahead. Protect yourself, your research and your co-workers.

B. STARTING UP YOUR BSC

1. Turn off the ultraviolet (UV) lamp, turn on the fluorescent light, and inspect the air intake grilles for obstructions and foreign materials. Remove any obstructions. Make sure the view screen window is adjusted (if adjustable) to the proper height (usually 10 inches).

2. Turn on the blower motor and allow it to operate for at least five minutes. This purges the air from the internal BSC cabinetry.

3. Wash your hands and arms with mild hand soap. Next, put on a rear fastening, long-sleeved gown with tight fitting cuffs. Put on a pair (or two pairs, depending on procedure and agent being used) of high quality latex gloves. Additional protection from contamination may be provided by wearing disposable sleeve protectors and a second or third pair of latex gloves. "Street" clothing should not be worn when using a BSC.

4. Next, disinfect the interior surfaces of the BSC by wiping down with appropriate disinfectant such as 1:10 Wescodyne, 1:10 bleach, or 70% alcohol. (Caution -- 70% Ethanol is highly flammable -- DO NOT USE in the presence of flame or spark)

5. Place a plastic-backed "chux-pad" or bench liner on the dry, disinfected work surface of the BSC. Avoid covering the air intake/exhaust grilles.

6. Install all necessary items for your experiment in the BSC at this time. Keep clean items segregated from dirty items (Figure 1).

   a. Minimize the amount of equipment and supplies; overloading the working zone with equipment and supplies may compromise the effectiveness of your BSC.

   b. Organize your material so that dirty "contaminated" items are not passed over (cross contaminate) clean items. Work from "clean" to "dirty" areas. A good work layout of materials would position clean items, i.e., pipettes, cultures, flasks, etc. toward the front or either side of the work surface. Place your waste container and contaminated pipette trays to the rear. You should work at least six inches back from the front of the air intake grille.

Figure 1. Recommended Layout of supplies and equipment to reduce contamination

7. If you are using a vacuum flask system in your BSC, put a vacuum protection device, a filter and flask, in line to protect the vacuum system.

8. After all equipment and supplies are added to your BSC, allow it to operate for an additional three to four minutes. This will allow the BSC to purge itself of airborne contaminants.

9. Assume a comfortable seating position in front of the BSC. Your chair should be adjusted to a comfortable height that promotes proper posture. When inserting your arms into the BSC remember that they are penetrating a delicate "curtain" of air. Allow the air curtain to stabilize around your arms before starting work. Avoid making rapid, jerking arm motions. Use smooth motions that avoid disrupting the air curtain.

10. Remember that the BSC air curtain is delicate and can only provide protection from contamination as long as it is not disrupted. The BSC is not a substitute for good

microbiological practices and does not automatically provide you with protection from potentially hazardous materials or automatically prevent contamination of the experiment and materials.

11. Follow good microbiological techniques, i.e., holding open tubes and bottles as horizontal as possible.

   a. Never mouth pipette. Use mechanical pipetting aids.

   b. Do not use vertical pipette discard canisters on the floor outside the BSC. Use horizontal pipette discard pans inside the BSC.

   c. It is not necessary to flame items. The flame creates turbulence in the airflow and will compromise sterility. If the lip of a tube or flask is wet, an aerosol may be created when the lip is flamed. Additionally, heat buildup can also damage expensive HEPA filters. Unattended burner pilot lights have created extensive fire damage to BSC's and sometimes entire laboratories.

12. If you need to introduce new items or remove items from the BSC, move your arms in and out slowly to minimize airflow disruption.

13. If you use equipment that creates air turbulence, such as a centrifuge, blender, or sonicator, place the equipment as far back in the BSC as possible (usually 1/3 of the way back from the front intake grill is acceptable). Stop other work while the equipment is operating.

14. Clean up all spills inside the BSC immediately and then wait 3-5 minutes before resuming work, if your laboratory operating procedures allow.

    An externally mounted drain valve is located under the drain pan of most BSC's. In the event of an accidental spill, you can remove large volumes of disinfected, spilled materials from under the work surface. The drain valve is not to be connected to a sink or similar drain. A bucket can be used to collect any spilled liquid for decontamination and disposal.

C. <u>CLEANING YOUR BSC</u>

1. When work has been completed, disinfect the exterior surfaces of potentially contaminated materials and supplies with appropriate disinfectant before removing them from the BSC. Remove all materials from the interior of the BSC.

2. Disinfect the interior BSC surfaces, including the inside of the view screen, with an appropriate disinfectant solution.

3. Allow the BSC to operate an additional 10 minutes, then turn the blower motor off. Caution; do not close the view screen when the blower is on, the view screen may crack or shatter.

   Note: Some BSC users prefer to leave the blower motor on continuously. This is a permissible practice. However, service life of your HEPA filters will be reduced.

4. Examine the spill pan beneath the work surface. Clean and disinfect the spill pan at least four times per year or as necessary.

   Be careful when removing interior work surfaces. They are heavy and may have sharp edges and corners. Consult your service manual or the Biosafety Officer for proper removal/maintenance procedures.

   Do not clean the spill pan when a BSC blower is operating. Paper towels may be accidently sucked into the airstream and can lodge in the blower motor and HEPA filter. Recovering paper towels can only be accomplished by decontamination and disassembly of the cabinet by an authorized service technician. Do not attempt retrieval yourself. Contact the Biosafety Officer for service.

5. Turn off the fluorescent lamp and turn on, if you wish, the ultraviolet lamp, if your BSC is so equipped.

6. Discard waste materials appropriately.

7. Remove your lab coat and gloves and then wash your hands thoroughly with a mild hand soap.

D. <u>FLAMMABLE OR EXPLOSIVE MATERIALS WITHIN BSC'S</u>

Standard electrical systems of Class II BSC's are not explosion proof. No flammable or explosive materials (chemicals, cleaners, or solvents) should be used in a BSC. Most BSC's are labeled with a warning sign on the front face of the cabinet warning against the use of flammable or explosive materials within it. Refer to your owner’s manual or call the Biosafety Officer for proper guidance. Under no circumstances should a chemical's vapor concentration be allowed to approach its level of flammability.

Each BSC type has its own ventilation characteristics. Remember, in the absence of a thimble exhaust duct connection, a BSC's air is recirculated within the laboratory. This can return a vapor or gas contaminant back to the "clean" work space and back into the laboratory until equilibrium is reached. Standard BSC HEPA filters are made of paper and do not remove chemical contaminants from the air. A chemical's equilibrium concentration level depends on the vapor or gas generation rate at the work space and the air exchange characteristics of the BSC and the laboratory facility.

HEPA filters remove only particulates. Gases and vapors will readily pass through HEPA filters and must be controlled by other methods. When planned work involves chemical carcinogens or solvents, it is necessary to evaluate the quantities to be used to determine the amount that might be entrained in the BSC air stream during an accident. By referring to the Threshold Limit Value Lists for chemical substances, from the American Conference of Industrial Hygienists and the Occupational Safety and Health Administration (OSHA), the proper method for dealing with the gases or vapors from chemical substances can be determined. Consult with Biosafety Officer.

BSC ASSIGNMENT FOR SPECIFIC BIOSAFETY LEVELS

The Office of Safety and Environmental Health (OSEH) generally recommends the Class II, Type A/B3 biological safety cabinet (BSC) for work with agents of low to moderate risk. This type of BSC is also recommended by the National Cancer Institute (NCI) for oncogenic agents of undefined hazard. A properly performance tested "certified" Class II, Type A/B3 biological safety cabinet will provide personnel, product, and environmental protection.

A thimble exhaust transition must be connected between the BSC exhaust plenum and the building exhaust system. The thimble exhaust transition eliminates BSC exhaust air recirculation within the laboratory.

BIOSAFETY CABINET TRAINING MATERIALS

The Biosafety Officer has several audiovisual programs for instruction of technicians, research investigators, supervisors, and others whose work involves biohazardous agents or materials requiring use of a Class II biological safety cabinet. Some of these audiovisuals are listed below:

A. *Effective Use of the Laminar Flow Biological Safety Cabinet* (16 minutes).

B. *Certification of Class II (Laminar Flow) Biological Safety Cabinets* (15 minutes).

C. *Formaldehyde Decontamination of Laminar Flow Biological Safety Cabinets* (13 minutes).

D. *Working Safely with HIV in the Research Laboratory* (20 minutes).

The programs can be viewed by appointment at the Office of Safety and Environmental Health (OSEH) or arrangements can be made to have the programs presented to groups at your laboratory.

E <u>REFERENCES</u>

1. *Laboratory Safety Monograph*, NIH.

2. Standard Number 49. *Class II (Laminar Flow) Biohazard Cabinetry*, National Sanitation Foundation, Ann Arbor, Michigan, Revised May, 1992.

3. Peterson, A.P.G. and Gross, E.E., *Handbook of Noise Measurement*, General Radio Company, Concord, MA 1984

4. *Industrial Noise Manual*, 3rd Ed., A.I.H.A., Akron, OH, 1975

5. *The Industrial Environment - Its Evaluation and Control*, U.S. Dept. of H.E.W., Center for Disease Control, N.I.O.S.H., Cincinnati, OH, 1973

6. *IES Lighting Handbook*, 5th Ed, Illuminating Engineering Society, New York, NY, 1972.

7. *Certification of Biological Safety Cabinets* Manual for the Harvard University Workshop sponsored by the National Institutes of Health, latest edition.

8. *NSF Listings Class II Biohazard Cabinetry*. National Sanitation Foundation. Ann Arbor, Michigan, February 1, 1993.

9. *Biosafety in Microbiology and Biomedical laboratories*, 3rd Edition. US Department of Health and Human Services and National Institutes of Health, Washington, DC, Revised May, 1993, Publication NO. (CDC) 93-8395.

10. *Laboratory Safety: Principles and Practices*, B.M. Miller (ed), American Society for microbiology, Washington, DC, 1986.

11. *Federal Standard 209E: Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones*, The Institute of Environmental Sciences, Mount Prospect, IL, 1992.

CHARACTERISTICS OF BSC'S AND OTHER AIRFLOW EQUIPMENT

Once you have assessed the risks inherent in your proposed laboratory program and having made a decision on protection objectives, you can make an appropriate equipment selection. There are three "Classes" of biological safety cabinets (BSC's) - Class I, Class II, and Class III. Class I and Class II are partial containment BSC's and are used with low to moderate risk agents. Class II is further divided into four "Types" with different configurations described below. Class III is an absolute barrier BSC and is used with high risk agents.

BSC's use high efficiency particulate air (HEPA) filters made of disposable, pleated filter medium installed with metal or paper separators, and encased in a rigid wood or metal frame. These dry-type HEPA filters allow passage of large volumes of air and trap 99.97% of thermally generated monodisperse dioctylphthalate (DOP) smoke particles sized 0.3 um. Particles larger **or smaller** than 0.3 um are trapped more efficiently. Therefore, HEPA filters block passage of aerosols containing viruses, bacteria, fungi and other biological agents (See Figure 1). HEPA filters should be annually performance tested "certified" to ensure proper function.

Figure 1. Typical HEPA Filter

A. CLASS I BIOLOGICAL SAFETY CABINETS

1. A partial containment, total exhaust biological safety cabinet (BSC) with HEPA filtered exhaust.

2. Class 1 BSC's provide personnel and environmental protection, but do not provide product protection (Figure 2).

3. Application: suitable for use with low to moderate risk agents where product protection is not needed.

4. Airflow:

   a. A constant flow of "dirty" unfiltered laboratory room air is drawn away from the operator into the cabinet front, thus providing personnel protection.

   b. The dirty air is drawn over the product and work surfaces. Hence, no product protection is provided by the Class I BSC.

   c. The dirty air is pulled into an exhaust HEPA filter where is cleaned or "polished", i.e., dirty particulate material is removed, thus providing environmental protection.

   d. The polished exhaust air is pulled into an electric motor/blower and sent into a duct or to be recirculated into the room.

Figure 2. Class I Biological Safety Cabinet

B. CLASS II, TYPE A BIOLOGICAL SAFETY CABINETS

1. Partial containment biological safety cabinet (BSC). Provides personnel, environmental, and product protection (Figure 3).

2. These BSC's maintain a minimum calculated average air inflow velocity of 75 feet per minute (fpm) through the work area access opening.

3. They are designed to either recirculate HEPA filtered exhaust air in the laboratory or exhaust the HEPA filtered air into a building duct thimble exhaust connection.

4. They may have internal positive pressure (potentially contaminated) ducts and plenums.

5. Type A BSC's are suitable for work with low to moderate risk biological agents in the absence of volatile toxic chemicals or volatile radionuclides.

6. Airflow:

   a. Constant air movement is split between the supply HEPA filter and exhaust HEPA filter.

   b. 30% of the total BSC air is exhausted through the exhaust HEPA filter.

   c. 70% of the total BSC air is recirculated through the supply HEPA filter into the work zone.

   d. Have a minimum intake air velocity of 75 FPM.

Figure 3. Typical Class II Type A Biological Safety Cabinet

C. CLASS II, TYPE B1 BIOLOGICAL SAFETY CABINETS

1. Formerly designated "Type 2".

2. A partial containment biological safety cabinet (BSC).

3. Provides personnel, environmental, and product protection.

4. Must be "hard connected" to the building exhaust system. Class II, Type B1 BSC's rely on the building exhaust system for proper operation.

5. These BSC's maintain a minimum average inflow velocity of 100 fpm through the work area access opening. They have HEPA filtered downflow air composed of 30% recirculated air.

6. They exhaust 70% of the contaminated downflow air through a hard connected exhaust duct after passing through an exhaust HEPA filter to the outside.

7. All internal biologically contaminated ducts and plenums are under negative pressure, or surrounded by negative pressure ducts and plenums. Hence, there is less potential for biological contamination of the laboratory.

8. Type B1 BSC's are suitable for work with low to moderate risk biological agents treated with small quantities of toxic chemicals or trace amounts of radionuclides. Work should be performed near the back of the work surface where interior air flows directly out of the cabinet to the exhaust system.

9 Air circulation through Class II, Type B1 BSC's is dynamic (Figure 4):

   a. Unfiltered laboratory room air enters the front of the BSC through the work access opening.

   b. The unfiltered air is drawn through the work access opening and into the front air supply air or "intake" grille.

   c. Then the unfiltered air is directed toward an electric blower motor located beneath the work surface. The blower motor is connected to a supply HEPA filter.

   d. The blower motor draws in the unfiltered air and pushes it out to the supply HEPA filter.

e. The unfiltered air passing through the supply HEPA filter has particulate material removed.

f. The clean HEPA filtered supply air is directed up positive pressure plenums along the sides of the BSC then over the top of the interior work surface.

g. The clean HEPA filtered supply air leaves the plenum and is directed through a diffuser which "straightens" the air so that it flows in a laminar fashion. The resulting vertical column of HEPA filtered laminar supply air descends through the work zone and passes over the product. At this point the air becomes "dirty" when it contacts the product.

h. Next, the vertical column of dirty supply air strikes the work surface and splits into two horizontally opposed air columns. One air column accounts for 70% of the original vertical column of air while the other air column accounts for 30% of the original column of air.

i. The 70% air column is drawn into the back supply air grille of the BSC's work surface and flows to an exhaust HEPA filter. Hence, 70% of this BSC's air is exhausted. The air is HEPA filtered and directed through a duct connected to the buildings' exhaust blower motor.

j. The other 30% air column is drawn into the front air intake grille where it combines with the unfiltered supply air entering the BSC from the laboratory. Thus, 30% of this BSC's air is recirculated through the supply HEPA filter.

10. OSEH does not recommend Class II, Type B1 cabinets, because of the requirement for a roof top exhaust HEPA filter and fan and difficulties associated with certification of the B1 cabinets.

Figure 4. Class II, Type B1 Biological Safety Cabinet

D. CLASS II, TYPE B2 BIOLOGICAL SAFETY CABINETS

1. A partial containment biological safety cabinet (BSC).

2. These BSC's provide personnel, product and environmental protection (Figure 5).

3. They are referred to as a "Total Exhaust" or "100% Exhaust" BSC's.

4. Type B2 BSC's must be "hard connected" to the building exhaust system. These BSC's rely on the building exhaust system for proper operation. The building exhaust system must be equipped with a bag-in/bag-out HEPA filter caisson to provide environmental protection.

5. These BSC's maintain a minimum average inflow velocity of 100 fpm through the work area access opening.

6. They have HEPA filtered downflow supply air. A blower motor takes in laboratory air and pushes it (positive pressure) through a supply HEPA filter. There is no recirculated air in this BSC;

7. All inflow and work area downflow air is exhausted, with no recirculation within the work area, to the outside through a hard connected building exhaust duct system after passing through a HEPA filter.

8. All internal contaminated ducts and plenums are under negative pressure, or surrounded by directly exhausted (non-recirculated) negative air pressure ducts and plenums.

9. Class II, Type B2 BSC's are suitable for work with low to moderate risk biological agents. They are used widely in toxicology laboratories and similar applications where chemical vapors are present. They must be used for biological agents treated with toxic chemicals and radionuclides.

Figure 5. Typical Class II, Type B2 (Total Exhaust) Biological Safety Cabinet

E. CLASS II, TYPE B3 BIOLOGICAL SAFETY CABINETS

1. OSEH recommends these BSC's for most research. They are classified as partial containment biological safety cabinets (BSC's).

2. These BSC's provide personnel, product, and environmental protection.

3. Type B3 BSC's can be "thimble connected" or "hard connected" to the building exhaust system.

4. A minimum average inflow velocity of 100 fpm is maintained through the work access opening.

5. HEPA filtered downflow air comes from a common exhaust plenum that is a mixture of downflow supply air and laboratory supply air.

6. All exhaust air is discharged to the outside after HEPA filtration.

7. All internal biologically contaminated ducts and plenums are under negative pressure, or surrounded by negative pressure ducts and plenums.

8. Type B3 BSC's are suitable for work with low to moderate risk biological agents treated with minute quantities of toxic chemicals and trace quantities of radionuclides that will not interfere with the work if recirculated in the downflow air.

9. OSEH recommends a "thimble" exhaust connection instead of a "hard" exhaust connection for most biomedical research applications.

Figure 6. Typical Class II, Type B3 Biological Safety Cabinet

F. CLASS III BIOLOGICAL SAFETY CABINETS

1. These BSC's are absolute barrier biological safety cabinets (BSC's).

2. They provide the highest level of personnel, product and environmental protection.

3. Class III BSC's are gas tight enclosures. Work within the BSC is accomplished through arm length rubber gloves attached to the front of the BSC.

4. Air change rates can be varied depending on what is necessary for rapid removal of aerosols created within the BSC. A minimum inflow velocity of 100 fpm and an internal negative pressure of not less than 0.5 inches (water gauge), is provided through any glove port in case a glove is accidentally punctured or detached.

5. Laboratory equipment such as a double door autoclave, fumigation chamber, dunk tank, or airlock must be directly attached to the BSC to allow safe introduction and removal of materials. Class III BSC's are usually custom designed to the user's special needs and requirements.

6. Class III containment can be compromised if gloves are punctured or by accidents that create a positive pressure within the BSC. Flammable solvents should not be used in these BSC's unless a careful evaluation has been made to determine that solvent vapor levels in the BSC will not reach hazardous levels (Figure 7).

7. This is suitable for work with high risk agents in an "open" laboratory.

Figure 7. Typical Class III Biological Safety Cabinet

G. OTHER CABINETS

There are a myriad of manufacturers offering a wide selection of models. Partial and absolute containment biological safety cabinets (BSC's) were discussed in the previous sections. This section is dedicated to cabinets not fitting the description of partial and absolute containment cabinets. These cabinets are generally not suitable for biological research.

H. HORIZONTAL LAMINAR FLOW CLEAN AIR BENCHES (CAB's)

CAUTION: Horizontal laminar flow clean air benches (CAB's) are not classified as biological safety cabinets (BSC's). These cabinets are not safe for work with chemical, radioactive, or biohazardous materials since they offer no protection to the operator or environment.

1. CAB's look very similar to a Class I BSC's in size and shape, but don't be fooled. CAB's are designed to provide only product protection; they do not provide any personnel or environmental protection (Figure 8).

2. The hallmark characteristic of a CAB is direction of airflow. Unlike Class I, II, or III BSC's, where airflow movement is from the front outside of the BSC to the inside of the BSC, CAB airflow moves in the opposite direction, toward the operator.

3. A CAB's blower motor pulls "dirty" air from the laboratory and forces it through a supply HEPA filter. The HEPA filtered air moves from the back of the work area forward to the product. Thus, the HEPA filtered clean air provides the product with protection from unwanted particulate materials. Once the air contacts the product it becomes "dirty" again. The "dirty air continues to flow out of the CAB and into the operator's face. Thus, aerosols generated by a material, product or process within the CAB are carried directly to the operator. Hence, the CAB does not provide personnel protection and must never be used with potentially biohazardous materials.

4. Originally, the CAB was designed to provide a clean air environment for the electronics manufacturing industry. CAB's are not recommended for biology or chemistry laboratories where cell culturing and/or viable agents are handled, because unknowing operators may mistake them for BSC's since they look similar.

5. CAB's have limited applications in quality control laboratories and aseptic assembly of sterile materials. This type of cabinet protects the work only and does not offer protection to the operator and/or the laboratory environment.

6. CAB's may occasionally be permitted by OSEH for work with materials such as germfree animals, pharmaceutical preparations, and sterile media preparations. They are never to be used with chemicals, radioactive materials or potentially infectious materials.

Figure 8. Horizontal Laminar Flow Clean Air Bench (NOT A BSC)

I. CHEMICAL FUME HOODS

Chemical Fume Hoods (CFH's) are not to be used with potentially biohazardous materials unless approved by the Biosafety Officer.

1. CFH's look similar to a Class I biological safety cabinet (BSC) in size and shape, but the CFH is a safety device designed specifically for personnel protection from potentially hazardous chemicals (Figure 8).

2. The proper technical slang word for the chemical fume hood is "hood".

3. The hallmark characteristics of a CFH are; the lack of HEPA filtration, the presence of externally mounted gas, vacuum, and air control knobs, and an external appearance which looks built-in or integrated with the existing lab cabinetry and bench work.

4. Airflow in CFH's is similar to Class I, II, and III BSC's, airflow movement is from the outside front of the CFH toward the inside.

5. CFH's cannot operate without being "hard-connected" to the building exhaust system. Typical CFH's do not have their own internal motor/blower system.

6. CFH's pull "dirty" air from the laboratory in over an airfoil (sill) through the view screen opening at a rate of approximately 100 fpm. Dirty air is then pulled over the work zone toward the back of the CFH. The enters an adjustable back wall baffle system with plenum and flows through a hard-connected building exhaust duct. The exhausted air continues through the building exhaust duct system where it mixes with other building exhaust air. The exhaust air is drawn through the building exhaust motor/blower system and is released to the atmosphere.

7. Sometimes the exhaust air is HEPA and charcoal filtered before release to the atmosphere so that aerosols generated by a product or process within a CFH are carried away from the operator and filtered before release to the atmosphere.

Figure 9. Chemical Fume Hood

BIOLOGICAL SAFETY CABINET USE GUIDELINES

A. Keep your laboratory and biological safety cabinet (BSC) meticulously clean. Minimize storage of boxes and supplies near a BSC.

B. OSEH recommends washing your hands and forearms thoroughly with mild soap and water for at least 10 seconds before and after working in your BSC. Wearing a clean gown and fresh latex gloves while working in a BSC increases your safety and helps reduce potential contamination of research materials or product.

Note: some publications recommend washing your hands with alcohol and not gloving while doing "tissue culture" work. OSEH does not recommend this practice because alcohol can dry out your skin. This may lead to dermatological problems. Furthermore, epithelial cells, with all associated microorganisms, can slough off your hands and create contamination problems with your cultures. Hence, you should wear latex gloves to protect yourself against contamination from potentially infectious materials and to protect your research material or product from contamination by your skin flora.

C. Keep your interior BSC work area clean and free of obstructions and debris. The effectiveness of a BSC is a function of directional laminar airflow (inward, downward, and outward) through high efficiency particulate air (HEPA) filters. Anything that disrupts airflow patterns (both inside and outside of a BSC) may increase the probability of contaminating your research material. BSC airflow patterns are disrupted by rapidly moving your arms in and out of the BSC, people walking rapidly behind you, down-drafts from the building supply air ventilation system, drafts from open laboratory doors or windows, a cluttered interior work space, and partially or completely blocked intake or exhaust grills.

D. Understand how your BSC works. Know your BSC's limitations. Plan your work ahead. Protect yourself, your research, your co-workers and the environment.

PURCHASING PROCEDURES FOR BIOLOGICAL SAFETY CABINETS

The following guidelines for purchasing ventilated cabinets, including Class II, Type A/B3/B1/B2 biological safety cabinets (BSC's) and laminar airflow clean air benches (CAB's), will help you make the appropriate cabinet decision for your clinical or research needs. All BSC and CAB purchases must be approved by the Biosafety Officer prior to ordering.

A. The National Sanitation Foundation (NSF) is a unofficial, noncommercial agency; not to be confused with the National Science Foundation. Its goal is to serve as a neutral medium in which business, industry, official regulatory agencies, and the public come together to deal with problems involving products, equipment, procedures, and services related to health and the environment. NSF is the national authority to which Class II biological safety cabinets (BSC's) are constructed and tested (NSF Standard No. 49).

B. Only NSF-49 listed BSC's will be approved for purchase and use within the Johns Hopkins Institutions. Call the Biosafety Officer for purchase approval details and a list of NSF approved BSC's.

C. One of the most critical NSF 49 standards are the "Biological Containment" tests. Biological tests are usually too complex to perform in-situ at most research laboratories. Biological containment tests assure that:

1. Aerosols produced by materials or work procedures within a BSC will be contained inside the BSC.

2. Outside contaminants will not enter the work area of the BSC.

3. Aerosols created within the BSC will not contaminate other equipment located within the BSC. These tests are the most critical tests for the evaluation of BSC safety and design. The success of these biological tests is dependent upon a proper balance between downflow air velocity, intake air velocity, and the height of the access opening. Air velocity measurements are determined for each qualifying BSC model. These measurements then serve as the reference for subsequent performance evaluations (certifications) by our outside contractor.

D. BSC's come in two widths, 4 foot and 6 foot. These are the inside, work area dimensions, not the outside dimensions. BSC's generally take up 5 or 7 feet of bench space, depending on whether they are 4 or 6 foot cabinets.

E. STEPS TO TAKE WHEN PURCHASING A BSC

1. The principal investigator contacts the Biosafety Officer and the Office of Safety and Environmental Health (OSEH) conducts a risk assessment of the proposed research.
2. The principal investigator registers their proposed research with the Biosafety Officer and the Institutional Biosafety Committee.

3. The Biosafety Officer recommends a Class and Type of BSC appropriate for the proposed research.

4. The purchase of a NSF 49 listed Class II, Type A/B3 BSC is usually recommended. However, other BSC Classes, and Types may be recommended for special conditions.

5. BSC's must be equipped with an OSEH-approved external exhaust duct transition system, such as a "thimble exhaust". However, the Biosafety Officer may temporally waive the thimble exhaust connection requirement. The principal investigator must request a temporary waiver in writing and describe the reasons for the request.

6. The requesting department must notify Facilities Management of their intention to purchase a BSC so that facilities can develop basic drawings and a general feasibility assessment of laboratory alterations involving the ventilated cabinet and thimble exhaust connection. The requesting department will benefit by asking for a rough estimate of installation costs from Facilities Management, instead of a detailed feasibility study with detailed mechanical drawings and airflow measurements.

7. Facilities Management will submit an installation feasibility assessment and BSC installation drawings to OSEH for review and approval. Facilities Management usually sends a cost quote to requesting department.

8. The requesting department completes a Purchase Request with all BSC and thimble exhaust transition information. The purchase request is sent to the Biosafety Officer for approval.

9. The Biosafety Officer sends the approved Purchase Request to the University Purchasing Department. Unapproved Purchase Requests are returned to the requesting department with an explanation.

F. OTHER CONSIDERATIONS WHEN PURCHASING A BSC

1. It is Johns Hopkins Institutions (JHI) policy for principal investigators to consult the Biosafety Officer prior to ordering, purchasing, moving, or installing ventilated cabinets, such as horizontal laminar flow clean air benches (CAB's) and biological safety cabinets (BSC's). OSEH will help you determine what Class and Type of cabinet is best for your research.

2. The Purchasing department sends all unapproved purchase requests back to the Biosafety Officer for review and approval.

3. The Biosafety Officer signs and dates the purchase request and sends it to the purchasing department for action.

4. Both the BSC and thimble exhaust transition should be purchased from the manufacturer. If a manufacturer does not offer an OSEH approved thimble exhaust transition, one can be fabricated by the university Facilities Management.

5. The purchase and installation of a BSC requires careful consideration. There are many BSC manufacturers that offer a wide variety of models to choose from. Each model has specific dimensions and performance specifications that should be known to the laboratory designer and researcher.

6. Be sure that the BSC you purchase is designed for your intended work. Sometimes CAB's must be sent back to the vendor for credit toward the purchase of a BSC.

7. All BSC's purchased must be listed in the most current National Sanitation Foundation (NSF) Standard 49 Listings. A copy of current listings is available from the Biosafety Officer.

8. When you are shopping for a BSC, insist on evidence from the manufacturer that the BSC has passed NSF Standard 49 personnel, product, and cross contamination biological tests. Such cabinets will be listed by NSF.

G. BEFORE YOU FILL OUT THE PURCHASE ORDER

After you have consulted with the Biosafety Officer and Facilities Management, you are ready to discuss price with the manufacturer's sales representative. It is a good idea to clarify the following points so there is no misunderstanding about the product details or delivery:

1. Make sure all arrangements are well planned in advance of the BSC's purchase and arrival.

2. Get a written price quote for the entire package, including the BSC Model number, optional equipment, thimble exhaust connection, etc. We recommend that you work out the details about shipping and delivery with the manufacturer's representative at the time of purchase. Get verbal agreements in writing.

3. Ask for a purchase discount. Some, but not all manufacturers and distributors provide Johns Hopkins with discount purchase incentives. Contact University Purchasing for details. Expect bigger discounts when buying multiple units.

4. Determine the costs of shipping and delivery.

   What is covered by the supplier?

   Are there special shipping requirements?

5. Remember, shipping and delivery may add significant additional costs to your purchase, depending on delivery location and level of difficulty.

6. Make sure that the sales representative clarifies in writing what "shipping and delivery" includes. Does delivery include moving the BSC from the receiving dock of your building to your laboratory? Does delivery also include BSC set-up in your lab? You should expect to pay additional charges for these extra services, such as uncrating and moving the BSC to the correct position in your laboratory, unless the representative includes these in writing on the bid.

   Note: Other options exist for moving BSC's from the loading dock to your laboratory, such as paying to have Facilities Management or moving contractors uncrate and move your BSC from the loading dock to your laboratory.

7. Confirm the Delivery Date.

8. Make sure the corridor pathways are clear for delivery to your lab.

   Will the BSC fit through door jams?

   Will the BSC travel around sharp, narrow corridors and corners?

9. Will the elevators in your building be able to accommodate this large, oversize package?

10. Does the BSC have to be brought up steps?

11. The Office of Safety and Environmental Health does not recommend moving this heavy equipment yourself. A professional moving service should be used. A delivered BSC package can weigh more than 1,200 pounds! Talk with your receiving department about delivery assistance to your laboratory.

    If necessary, coordinate lab delivery with a bonded professional moving company.

    You must let the moving contractor know that you want the BSC lifted onto its stand or leg extensions (working position), because the mover will need to bring a hydraulic lift for this purpose.

    If your department decides not to use a moving contractor, the department must assume the risk of possible damage to the BSC and potential injury to personnel incurred by uncrating and moving this equipment! OSEH does not recommend this procedure.

12. When negotiating the price of your BSC, make certain that you include the cost of the required thimble exhaust connection described in a later section of this manual.

H. BIOLOGICAL SAFETY CABINET (BSC) ARRIVAL DAY

1. When the BSC package arrives, inspect it carefully. Compare the invoice with the delivered package. Check for any damage or missing materials and report them immediately to the proper authorities regardless of how insignificant they may first appear. Be careful of sharp crating material and let the loading dock personnel help you check for damage.

2. Call the Biosafety Officer to arrange for certification after the BSC is installed by facilities. Call facilities to let them know that your BSC has arrived and is ready to be connected to your laboratory plumbing, electrical, and supply/exhaust air ventilation systems.

VENDORS OF BIOLOGICAL SAFETY CABINETS

Manufacturer Address / Phone Number

Baker Baker Company, Inc.
Sanford Airport
Sanford ME 04073
(207) 324-8773

LOCAL BAKER DISTRIBUTER:
GTS Scientific, Inc.
P.O. Box 7555
Gaithersburg, MD 20898
(301) 929-1444

Bellco Bellco Glass, Inc.
340 Edrudo Road
Vineland, NJ 08360
(800) 257-7043

Envirco Envirico, Inc.
6701 Jefferson NE
Albuquerque, NM 87109
(505) 345-3561

Forma Scientific Forma Scientific, Inc.
P.O. Box 649
Mill Creek Road
Marietta, OH 45750
(800) 848-3080
LOCAL FORMA DISTRIBUTER
(800) 872-7133 ext. 4648

Germfree Germfree Laboratories, Inc.
7435 N.W. 41st Street
Miami, FL 33166
(305) 592-1780

Labconco Labconco Corporation
8811 Prospect
Kansas City, MO 64132
(816) 333-8811

LOCAL LABCONCO DISTRIBUTER:
504 Sunfield Way
Frederick, MD 21702
(800) 821-5525

Microzone (Formerly Canadian Cabinets Company, Ltd.)
25F Northside Road
P.O. Box 11336, Station H
Nepean, K2H7V1 Canada

Nuaire Nuaire, Inc.
2100 Fernbrook Lane
Plymouth MN 55447
(612) 553-1270

LOCAL NUAIRE DISTRIBUTER:
LabRepco.
626 Street Road.
Southampton, PA, 18966
(800) 521-0754

BIOLOGICAL SAFETY CABINET INSTALLATION GUIDELINES

A. BEFORE GETTING STARTED

1. Ask the Biosafety Officer to review your work protocols and approve all clean air bench (CAB) and biological safety cabinet (BSC) and associated ventilation system installations, moves, and modifications to existing installations - before proceeding with the work.

2. The Biosafety Officer must provide a "Clearance" for any ventilated cabinet maintenance. A "Clearance" sign must be posted on the ventilated cabinet so personnel know it is safe to proceed with work. No work is permitted on ventilated cabinets or associated ventilation systems unless clearance has been given.

3. CAUTION: Modifications to biological safety cabinets (BSC's) and clean air benches (CAB's), including drilling holes in the outside cabinet, or changes to the blower speed or air velocity are not permitted. Contact the Biosafety Officer if any accidental or intentional changes are made to any ventilated cabinet.

4. Unauthorized ventilated cabinet modifications may affect certification and/or seriously affect containment performance. Additionally, the BSC manufacturer's warrantee may be voided.

B. BIOLOGICAL SAFETY CABINET GENERAL INFORMATION

1. All utility services supplied to BSC's must be readily accessible by authorized service and maintenance personnel. All service utilities installed on a BSC shall conform with all applicable building codes, electric codes, and university guidelines.

2. The specifications for each BSC manufacturer/model are different! Review the manufacturer's specifications prior to installation to insure equipment compatibility. Call the Biosafety Officer for additional information.

C. SET-UP LOCATION

Air turbulence can cause disruption of a BSC's containment air curtain. This can cause contamination of your materials. When selecting the location for your BSC in your lab, air turbulence sources within the laboratory should be identified and avoided.

Examples of sources that cause air turbulence are ventilation air supply inlets that could blow across the work access opening or onto the BSC exhaust filter, open windows, and open doors.

Locate your BSC away from pedestrian traffic. People walking close to your BSC can create enough air turbulence to cause a disruption of the work opening air curtain.

Ideally, the selected BSC location will have convenient building utility service connections for electricity, vacuum, gas, and exhaust air. Contact your facilities management office for assistance.

The diagram below illustrates optimal locations for BSC installations. The final installed position must be approved by the Biosafety Officer.

REMINDER - BSC's generally take up 5 or 7 feet of bench space, depending on whether they are 4 or 6 foot cabinets.

Figure 11. Suggested BSC Locations for a Typical Laboratory

D. <u>GAS AND VACUUM UTILITY CONNECTIONS</u>

1. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

2. Where space permits, a 6-inch (152 mm) clearance should be provided behind and on each side of the BSC to accommodate utility gas and vacuum piping. If not feasible, the Biosafety Officer may approve a minimum 3-inch (76 mm) clearance on each side and rear.

3. An easily accessible emergency gas shut-off valve must be installed externally on the supply gas line. The emergency gas shut-off valve must be equipped with an attached valve handle. A pipe union must be installed between the emergency gas shut-off valve and the BSC petcock line (Figure 12).

4. Utility piping should not be attached, hung, suspended, or supported, by the ventilated cabinet. Unauthorized cabinet modifications can seriously damage its operation. Install all piping in accordance with all appropriate building codes.

Figure 12. Typical Emergency Gas Shut-Off Valve Installation

E. <u>ELECTRIC SERVICE CONNECTIONS</u>

1. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

2. Interior BSC Service: Only authorized maintenance personnel should service interior BSC electronics. Contact the Biosafety Officer to arrange for service.

3. Exterior BSC Service: Electric connections for BSC's should be accessible to authorized service personnel and to permit electrical safety testing without having to move the cabinet.

F. <u>NEW INSTALLATIONS</u>

1. Refer to the manufacturer's specifications for new installation procedures and requirements. Various manufacturers of BSC's specify different plug types, numbers of power cords used, voltages, and amperage ratings. Contact the Biosafety Officer for assistance with manufacturer specifications.

2. All electric service connections should be made in accordance with applicable building codes.

G. WORKING HEIGHT

1. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

2. When a new BSC is installed, it is necessary to determine the proper work height before the BSC is moved into its final location. The height at which a BSC work surface is set is termed the "work height". The work height chosen by you will affect the overall height of the BSC. This will affect laboratory utility connection locations and the operator sitting height.

3. "Bench-Top" BSC work heights manufactured to be adjustable. Most bench top BSC's can be adjusted to about 30 to 36 inches above the floor. The 30 inch work height is the traditional sit-down height. The 36 inch work height is the traditional stand-up height.

4. "Console" BSC's do not have adjustable work heights. Console models are manufactured to a preset height and can only be adjusted about an inch or two to compensate for variations in the floor.

THIMBLE CONNECTION EXHAUST AIR VENTING

A. BACKGROUND

According to Johns Hopkins Institutions (JHI) policy, biological safety cabinet (BSC) HEPA filtered exhaust air must be removed from the laboratory via a "thimble exhaust connection" (TEC) attached to the building exhaust air system.

B. THIMBLE EXHAUST CONNECTION (TEC) GENERAL DESCRIPTION

1. A "tepee shaped" canopy hood with frontal access door to allow certification and maintenance (Figure 13).

2. The function of a TEC is to connect the building exhaust system to the BSC in the laboratory. The TEC draws 100% of the BSC exhaust air, which eliminates BSC exhaust from the laboratory, along with an additional 15-50% laboratory room air. The airflow in the exhaust duct connected to the thimble must capture the equivalent of **150 %** of your BSC's rated exhaust volume. Therefore, both laboratory air and BSC air is exhausted.

3. The TEC should be positioned directly above the BSC exhaust air plenum, but the TEC is not directly attached to the BSC. The hallmark TEC characteristic is an **air-gap** between the TEC lower edges and the top of the BSC exhaust air plenum. Hence, the thimble is "soft-connected" to the BSC's exhaust plenum;

4. Sometimes, an extended "collar" transition piece must be fitted around the BSC exhaust air plenum. This acts as an extension of the exhaust air plenum and helps direct the BSC's exhaust air through the air-gap and into the TEC canopy.

5. The design of the exhaust system must be based on the unique and individual set of conditions for each BSC installation. The building exhaust system must maintain an exhaust volume within limits of the ventilated cabinet manufacturer's specifications.

6. Adjustable dampers should be provided with all exhaust systems to allow exhaust volume adjustment. For special laboratory installations such as Biosafety Level 3 (BSL-3) laboratories, a 100% shut off damper must be provided so that effective BSC and/or laboratory decontamination can be achieved.

7. Whenever possible, each BSC should be connected to a separate building exhaust system of its own (dedicated exhaust system). However, it is usually more economical to manifold BSC exhausts together.

Figure 13. Thimble Exhaust Connection

C. RESPONSIBILITY FOR FABRICATING THE EXHAUST CONNECTION

Facilities is responsible for fabricating and connecting the TEC to the building exhaust system(s) in conformance with local building codes and manufacturer specifications. The Biosafety Officer evaluates design(s) and approves them prior to fabrication and installation by facilities. The requesting department is generally responsible for all costs associated with connection of the BSC thimble to the building exhaust system.

D. THIMBLE EXHAUST CONNECTION SAFETY ADVANTAGES

For Class II, Type A BSC thimble exhaust connections (TEC's) provide an additional safety factor for your laboratory.

1. Potentially hazardous airborne chemical vapors and gases are eliminated through the TEC and associated building exhaust system instead of being exhausted into laboratory. Thus, personnel and potentially susceptible work is protected from inadvertent chemical exposure.

2. A commonly used chemical for BSC decontamination is formaldehyde gas. Formaldehyde is a known carcinogen. The TEC and associated building exhaust system minimizes exposure of research materials, people and animals to formaldehyde gas because the gas is exhausted from BSC into the building exhaust system rather than allowing it to be exhausted into the laboratory.

3. Heat generated by BSC's, centrifuges, freezers and other equipment can elevate laboratory temperatures. The TEC is an important way to exhaust heat generated in the laboratory.

4. TEC's provide protection to your laboratory environment if the BSC exhaust HEPA filter seals fail or are accidentally damaged.

   Note: All Class III, and Class II, Type B1, B2, and B3, BSC's require a mandatory "hard" exhaust connection and will not operate without a solidly attached (no air gap) building exhaust system.

E. BSC SUPPLY AND EXHAUST AIR VOLUME REQUIREMENTS

1. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

2. The volume of air exhausted through a properly operating BSC must be taken into consideration when designing the air balance (supply air and exhaust air volumes) for a laboratory. Approximate values of required exhaust air and therefore make-up supply air volumes for BSC's are presented in a later Section of this Manual.

3. Required face velocities (and thus, the requirements for make-up "supply" air volume) for BSC's may vary with each application. Contact the Biosafety Officer for approval.
4. Performance specifications for Class II BSC's, types A and B, are firmly established; the exhaust air volumes for these BSC's should always be very close to those given by the BSC manufacturer.

F. <u>TEC INSTALLATION PROCEDURES</u>

1. Bench top BSC's that you may purchase will have varying heights ranging from 89 to 91.5 inches. Therefore, clearance distances must be planned in advance of installation.

2. Console BSC's that you may purchase will have heights ranging from 79.4 to 85.6 inches (they are shorter).

3. The OSEH approved TEC design has a height ranging from 22 to 24 inches, measured from the top of the BSC to the top of the exhaust duct connection. Therefore, an OSEH-approved TEC will fit all console models in laboratories with nine foot high ceilings. A dimensional drawing is available from the Biosafety Officer.

4. TEC Purchase Requirements:

   a. Use a low profile TEC manufactured by Baker, Nuaire, or other OSEH approved manufacturer in laboratories with ceiling heights less than 9.0 feet in order to avoid dimensional clearance problems.

   b. TEC installations must be approved for by the Biosafety Officer.

   c. In some cases, custom TEC's must be fabricated. Custom TEC's must have the OSEH-approved air-break design.

   d. TEC's must be designed so that they fasten to the BSC in such a way as to allow annual certification of the BSC without movement or disassembly of the thimble, duct, or BSC.

   e. The BSC purchaser is responsible for the cost of TEC and associated building exhaust system connections. Contact your facilities department for information.

5. Custom Installations:

Specifications for thimbles and 100% shut off dampers for special situations, such as biosafety level 3 (BSL-3) laboratories with low ceilings, must follow these guidelines:

a. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

b. The airflow in the exhaust duct connected to a low profile TEC must capture the equivalent of 120% of a BSC's exhaust volume. Different manufacturers produce TEC's with differing specifications. Contact the Biosafety Officer for additional information.

c. The combined thimble and 100% shut off damper assembly must be pressure tested to withstand at least 2 inches (water gauge) pressure without leaking.

d. The BSC + thimble + 100% shut off damper combination must pass performance tests as specified in the National Sanitation Foundation Standard 49. It must pass personnel, product and cross contamination challenges and NSF-49 performance specifications.

e. The BSC + thimble + 100% shut off damper combination can be installed in a laboratory with a nine-foot ceiling. Your BSC working height should be set at the 30-inch work position.

G. TEC SPECIFICATIONS AND SHOP NOTES

1. Do not perform any work on the BSC or associated exhaust ducting unless you have received clearance by the Biosafety Officer for the current work. A signed and currently dated maintenance clearance form must be posted before maintenance or service can proceed.

2. Do not modify the existing BSC. Do not bend, drill into, cut into, nail into, screw into, hammer, weld, etc., without approval of the Biosafety Officer.

3. All sharp edges must be filed round.

4. Provide an adjustable volume damper in the building exhaust duct.

5. Protect the BSC's exhaust filter from damage. Anything dropped onto an exhaust HEPA filter will cause damage.

6. Fabricate the access door as large as possible. The access door shall be installed toward the BSC front to facilitate future service and BSC certification procedures. The hinge shall be a minimum of 12 inches above the top of the collar (Refer to Figure 14).

7. The TEC access door shall be fabricated with a 1-inch flange on each side that will fit into the thimble "s-slip" bend.

8. Dimensions between associated thimble components must be followed.

9. Fabricate the thimble and collar from appropriate sheet metal.

10. The thimble exhaust connection shall be sized to cover the entire exhaust HEPA filter plenum plus 2 inches in each dimension (Refer to Figure 14, "F").

11. A discharge collar shall be installed on the BSC exhaust plenum if not already provided. Attach the discharge collar with existing BSC studs (Refer to Figure 14, "C").

12. Silicone seal all joints on collar and thimble exhaust duct.

13. Stand offs are attached to the thimble exhaust base only. Do not attach them to the BSC.

14. Every thimble installation shall be inspected and approved by the Biosafety Officer.

Figure 14. Thimble Exhaust Connection Drawing and Specifications

A - THIMBLE EXHAUST CONNECTION AND EXHAUST DUCT
B - HINGED THIMBLE CONNECTION SERVICE ACCESS DOOR
C - BIOSAFETY CABINET DISCHARGE COLLAR
D - THIMBLE "S-SLIP" BEND TO ACCOMMODATE ACCESS DOOR FLANGE
E - THIMBLE CONNECTION SERVICE ACCESS DOOR PIANO HINGE
F - PROPER THIMBLE CONNECTION COLLAR SPACING DIMENSIONS

HARD CONNECTION EXHAUST AIR VENTING

A. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

B. Class II, Type B1, B2, some B3 biological safety cabinets (BSC's), and all Class III BSC's require a building exhaust system directly connected (hard connected with no air gap) to the BSC's exhaust plenum. This usually requires an individual exhaust duct and blower (See Figure 15).

C. The building exhaust system flow rate must be professionally adjusted to the BSC manufacturer's specifications (the BSC will not operate without the building exhaust air volume properly adjusted). Contact your facilities department and the Biosafety Officer for more information.

D. The hard connection thimble has the following characteristics:

A transition piece which connects the building exhaust system with a BSC must have the following characteristics;

1. Be constructed of seamless sheet metal.

2. The design must not permit an "air-gap" between the BSC and the building exhaust connection. The transition must be attached directly to the BSC's exhaust air plenum using existing studs and a leak proof gasket. The transition must be secured to the building exhaust duct system with a seamless connection.

3. The hard connection must be equipped with an air-tight inspection door and a plugged access hole for periodic velocity tests.

Figure 15. Typical BSC Hard Exhaust Connection

FREQUENCY OF CABINET CERTIFICATION

A. All ventilated cabinets must be at least annually performance tested or "certified" in accordance with manufacturer specifications. The certifications will follow the NSF-49 Standard (for BSC's) or the Federal Standard 209E (for CAB's).

B. Appointments for certification are obtained by calling the Biosafety Officer.

C. Frequency of testing is not limited to once a year. Ventilated cabinets may be certified as often as required.

D. Payment for certification must be made at the time that you schedule your service with the Biosafety Officer.

E. Performance tests/certifications must be conducted only by the OSEH-authorized Johns Hopkins Institutions contractor.

F. Ventilated cabinet installations associated with new construction or renovation project specifications should include the statement that after installation, each ventilated cabinet must be performance tested to the appropriate performance standard (NSF or 209-D) and be certified to the manufacturer's design performance specifications.

G. Performance testing / certification must be performed:

1. Before initial use.

2. After moving a BSC or CAB from one location to another.

3. After replacement of high efficiency particulate air (HEPA) filter(s). BSC HEPA filter replacement can be expected to occur at intervals of every 3 to 5 years.

4. At least annually.

5. After possible BSC or CAB damage.

6. Following a large spill or accident inside the BSC.

7. When requested by the Biosafety Officer.

ANNUAL CABINET LABOR AND SERVICE CONTRACT

A. The Office of Safety and Environmental Health (OSEH) has arranged for all Johns Hopkins Institutions ventilated cabinets to be certified at least on an annual basis through one approved contractor (sole provider of services).

B. OSEH and Corporate Purchasing negotiate with qualified external contractors for the JHI sole provider of services contract. The contract is awarded to the most competitive bid. Hence, individual JHI ventilated cabinet user costs are much lower than those available to the general industry.

C. The certification and service repair costs are charged to the individual ventilated cabinet user via;

1. Hospital: Purchase Order number payable to the contractor; or

2. University: Materials and Services Request made payable to the Office of Safety and Environmental Health (OSEH).

D. The Certification and Service Contract:

1. Includes certification and the associated performance evaluation reports for the BSC owner and OSEH.

2. Does not require additional charges for labor if a repair is needed. Labor charges usually cost about $60/hr.

3. Does not require additional charges for replacement parts (except HEPA filters, and pre-filters) which break under normal use.

4. Extra cost charges include; replacement parts broken due to negligence, extra formaldehyde gas decontamination services, custom installation of accessories, and fabrication of parts.

E. The Biosafety Officer oversees all work conducted by the contractor. Each BSC is performance evaluated under the provisions and guidelines of the National Sanitation Association Standard 49 (NSF 49). Each CAB is performance evaluated under the provisions and guidelines of Federal Standard 209E.

F. You are encouraged to watch the certification testing procedures, and to review the test results which are given to you by the certifier.

SCHEDULING CABINET SERVICE AND CERTIFICATION

A. The Biosafety Officer sends reminder notices to all ventilated cabinet owners and users about a month before the due date for annual performance evaluation and certification. Reminder notices will continue to be sent until a response from the owner has been received by the Biosafety Officer.

B. PAYMENT: The Office of Safety and Environmental Health (OSEH) must receive either a completed "Materials and/or Services Request" (Form B-26) made payable to OSEH; or a valid Purchase Order number (PO#) made payable to the contractor, **before** the Biosafety Officer will schedule the certification.

Please allow approximately two weeks lead time to schedule your ventilated cabinet certification and service.

C. Ventilated cabinets which have not been certified within one month after the anniversary date will be posted with a yellow warning sign "**This Cabinet Is Not Certified For Use With Biohazardous Agents**". Those cabinets which remain uncertified will be removed from the maintenance contract. Cabinets not on the service contract will only be serviced at the considerably higher "individual" retail rate, which be two or three times more expensive than the contract rate.

Figure 16. Sample of a Completed M&S Form

MOVING YOUR CABINET

It is a common practice to move laboratory ventilated cabinets to other locations within the lab or to other laboratories. However, despite the apparent simplicity of the job, there are certain conditions that must be met prior to moving this equipment. These guidelines were developed to insure the safety of people who work with ventilated cabinets.

A. Ventilated cabinets must not be moved unless the Biosafety Officer has approved the new location and "cleared" the cabinet for moving. Obtain the proper "Clearance for Maintenance" from the Biosafety Officer, and when necessary, Radiation and Chemical Safety Officers.

B. Existing ventilated cabinets and ancillary equipment, such as thimble exhaust ducting, gas, electric and vacuum systems, must be "CLEARED FOR MAINTENANCE" by the Biosafety Officer prior to disassembly.

C. Contact your Facilities Management office for instructions on how your ventilated cabinet will be physically moved.

D. Prior to a move, **all** biological safety cabinets (BSC's) must be professionally decontaminated with formaldehyde gas. Call the Biosafety Officer for scheduling and payment details.

E. After the ventilated cabinet is moved; it must be recertified according to applicable performance standards. Call the Biosafety Officer for scheduling and payment details.

CERTIFICATION TESTS

A. Call the Biosafety Officer to schedule routine, priority or emergency service or certification of your ventilated cabinet equipment.

B. Provide the Biosafety Officer with an appropriate Purchase Order number (PO# for Hospital) or Materials and Services Request Form (M&S for University) as described below:

1. M & S's should be filled out so that they are similar to the sample (Figure 16). Include the cabinet model number, serial number, room number location of the cabinet, account number, date, phone number, and name of the person to call to make an appointment for certification.

2. If you are using a PO # for service or certification, make it payable to the contractor. Call the Biosafety Officer to get the name of the contractor. You will be invoiced directly by the contractor.

3. Send the completed Materials and Services Request Form to OSEH.

4. The Biosafety Officer will coordinate all service with the contractor. DO NOT CALL THE CONTRACTOR DIRECTLY.

5. Allow at least 2 weeks for service unless special arrangements have been made in advance with the Biosafety Officer.

C. BIOLOGICAL SAFETY CABINET (BSC) PERFORMANCE EVALUATION TESTS

The National Sanitation Foundation (NSF) is not an official agency and is not a commercial agency. Its' goal is to serve as a neutral medium in which business and industry, official regulatory agencies, and the public come together to deal with problems involving products, equipment, procedures, and services related to health and the environment. NSF acts as the national authority to which Class II biological safety cabinets are constructed and tested. The NSF-49 performance tests are listed below for your information:

1. AIR VELOCITY TEST

This test is performed to measure the velocity of downward airflow.

2. SUPPLY AIR VOLUME TEST

3. WORK ACCESS OPENING AIRFLOW TEST

This test is performed to determine the face velocity of inflow air (supply air) through the work access opening, and the calculated exhaust flow volume.

4. HEPA FILTER LEAK TESTS

This test is performed to determine the integrity of supply and exhaust HEPA filters, filter housings, and filter mounting frames.

5. AIRFLOW SMOKE PATTERN TEST

This test is performed to determine that the airflow along the entire perimeter of the work access opening is inward, airflow within the work area is downward with no dead spots or refluxing, ambient air does not pass on or over the work surface, and there is no refluxing to the outside at the window wiper gasket and side seals.

6. ELECTRICAL GROUND FAULT TEST

This test is performed to determine the electrical leakage and ground circuit resistance to the BSC ground connection, and determine if a potential shock hazard exists.

7. NOISE LEVEL TEST

This test is performed to provide a uniform method for measuring noise levels produced by the BSC.

The need for such a test is based on belief that workers should not be exposed to unnecessary noise. Secondarily, an increase in noise level is a indication of mechanical deterioration or malfunction.

8. ULTRAVIOLET LIGHT INTENSITY TEST

Ultraviolet (UV) lighting, when requested by the purchaser, shall be installed so that it does not reduce the required performance of the BSC. The UV irradiation must be in accordance with American Conference of Governmental Hygienists (ACGH) standards, and the irradiation must not affect any of the construction materials or containment integrity of the BSC.

a. For decontamination purposes, low pressure mercury vapor lamps which emit about 90% of their radiation at 254 nm, are generally used. Cold cathode, low ozone lamps, are preferred.

   Hot cathode lamps, Type G, could be used where space limitations are imposed by equipment design.

b. UV lamp replacement is provided by the contractor free of charge as part of the institution's service contract. Call the Biosafety Officer for replacement or service.

c. UV Lamp Safety Considerations:

   Exposure of your eyes and skin to direct or strongly reflected UV radiation should be avoided. The effect of UV radiation depends on irradiance level, wavelength, part of body exposed, and individual sensitivity. Overexposure of the eyes results in a painful inflammation of the conjunctiva, cornea, and iris. Symptoms develop 3 to 12 hours after exposure and usually disappear in a few days. Overexposure of the skin produces a reddening (i.e., erythema) in 1 to 8 hours after exposure.

   UV exposure should be minimized as a matter of good practice; eyes should be protected by UV-opaque glasses with side shields and the skin should be protected by cloth or rubber coverings.

d. This test is performed to determine the intensity of ultraviolet (UV) radiation, in microwatts per cm^2, on the work surface in the BSC. This test will determine if the

UV lamps are providing sufficient UV radiation for decontamination purposes. A secondary function of the test is to clean the lamps so they operate more efficiently.

HAZARD SIGNAGE

A. PURPOSE

A hazard warning signage system for research and diagnostic laboratories (Copyright 1995, Johns Hopkins University) has been designed to bring uniformity to the hazard warning signs used in The Johns Hopkins Institutions. This guide describes the signage system and sets the conditions under which the signs are to be posted. It is important that all employees and visitors comply with the policy for entering areas where these signs have been posted.

B. DESCRIPTION

The hazard warning signs illustrated in this section are to inform personnel and visitors that a hazard exists in the area. Three (3) levels of risk have been established. The degree of hazard is indicated by the admission instructions on the placard. The specific hazard is identified by symbols and/or hazard warnings affixed to the placard.

The levels of risk are defined on the admittance placards as follows:

1. **CAUTION - ADMITTANCE TO AUTHORIZED PERSONNEL ONLY**. (Exhibit 1). Visitors and personnel not assigned to the area must secure permission to enter from the investigator, supervisor or administrator in charge of the area.

2. **CAUTION - RESTRICTED AREA - ADMITTANCE TO AUTHORIZED PERSONNEL ONLY** (Exhibit 2). Admittance is forbidden to all except those assigned to the area unless accompanied by the principal investigator or laboratory supervisor. This sign shall also be used to identify specific higher risk locations, i.e., incubators, refrigerators, biological safety cabinets, within areas posted with a restricted area sign.

3. **DANGER - DO NOT ENTER - CONTAMINATED AREA** (Exhibit 3) . This no-access sign is to be used in temporary situations, such as following an accident. Areas posted with this sign shall be off limits to all personnel except the investigator who posted the sign. The sign shall be taken down immediately after the source of danger is removed.

The signs posted at accesses to laboratories and other facilities will be 10" X 10" placards.

Pressure sensitive labels identifying the type of hazard will be affixed to the placard. The labels available for hazard identity - hazard symbols, hazard warnings, personal protective equipment requirements, and required work practices - are shown in Exhibit 4. If more than one hazard exists in an area, the symbols should be displayed on one

placard. The access restriction shall be determined by the greater hazard. Specific safety instructions can also be affixed to the placards. Smaller, 5" X 5", adhesive backed warning signs, will be provided for identifying hazardous locations within laboratories.

The text accompanying Exhibits 5 through 14 specifies the conditions under which the most frequently used hazard warning signs will be posted. For simplicity, the warning signs illustrated show only the hazard symbol or warning affixed to the placard indicating the level of risk. The specific precautions in the guidelines accompanying each illustration shall apply whenever the symbol or hazard warning is affixed to the corresponding placard by itself or together with other hazard warnings.

C. RESPONSIBILITY

It is the responsibility of the investigator to determine the need for hazard warning(s) and to contact the Office of Safety and Environmental Health to determine the level of hazard in the area. The Office of Safety and Environmental Health will maintain records of all areas posted.

It is the responsibility of the investigator (or administrator) to list the names and telephone numbers of two individuals on the admittance placard as emergency contacts. The individuals listed must have some familiarity with the hazards in the posted location.

D. AVAILABILITY

The Office of Safety and Environmental Health will maintain the supply of warning signs and labels.

Exhibit 1

Exhibit 2

Exhibit 3

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Exhibit 4

HAZARD WARNING LABELS

When posted this **Restricted Area** label signifies "admittance to laboratory personnel only", all others must obtain permission from the laboratory supervisor to enter the area or to open the containment equipment labeled as restricted.

This **biohazard symbol**, without a specific hazard description is a general biohazard warning to be used where there are multiple biological hazards, where biological wastes are stored, and for mixed biological waste containers.
This label, with the restricted area label is generally used to identify refrigerators, incubators, and cabinets where biological agents or materials are stored.

Rooms or areas (research and clinical laboratories, certain hospital unit laboratories and utility rooms) where human body fluids, unfixed cell tissue or organ cultures are handled for research, diagnosis, shipment and equipment used for research or diagnosis with specimens containing **potentially infectious materials (PIMS)** shall be conspicuously posted with the **Biohazard Symbol** and the **Caution - Potentially Infectious Materials** warning.

Laboratories and support areas where viral, bacterial, rickettsial, fungal, and parasitic agents requiring containment at biosafety level 2 or greater are used or stored shall be conspicuously posted with the **Caution - Infectious Agents** sign.

Animal rooms and other containment areas which house animals deliberately infected with agents requiring BSL 2 (or greater) containment shall be posted with the **Biohazard Symbol** and the **Caution - Infected Animals** warning.

This **Biosafety Level 2 (BSL 2)** label, with the appropriate biohazard warning, designates a containment level which meets the BMBL* recommendations for BSL 2 work practices, safety equipment and facility design.

This **Biosafety Level 3 (BSL 3)** label, with the appropriate biohazard warning, designates a containment level which meets the BMBL* recommendations for BSL 3 work practices, safety equipment and facility design.

*CDC/NIH 1993 Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition. U.S. Department of Health and Human Services, Public Health Service, and subsequent revisions

This **Biosafety Level 3** - **Work Practices** label, with the appropriate biohazard warning, designates a BSL 2 facility in which **special work practices**, the additional practices recommended for BSL 3, are required.

Each area or room in which radioactive **materials** are used or stored or where radioactive wastes are accumulated or stored shall be conspicuously posted with a sign bearing the **radiation symbol** and the **Caution - Radioactive Materials** warning.

Each radiation area (any area, accessible to personnel, in which there exists radiation at such levels that a major portion of the body could receive in any 1 hour a dose in excess of 5 millirem or in any 5 consecutive days a dose in excess of 100 millirem) shall be conspicuously posted with the **Caution - Radiation Area** sign.

Each high radiation area (any area accessible to personnel, in which there exists radiation at such levels that a major portion of the body could receive in any one hour a dose in excess of 100 millirem) shall be conspicuously posted with the **Caution** - **High Radiation Area** sign.

Areas, accessible to personnel students or visitors, generating microwave energy in excess of 10 mW/cm^2 averaged over 0.1 hour shall be posted with the **microwave radiation** sign.
This warning sign is **not** intended or necessary for domestic type microwave ovens.

All rooms or areas, accessible to personnel, in which there exists a potential for exposure to **ultraviolet light** above the NIOSH published recommended exposure limit (REL) for occupational exposure. At 254nm, the wavelength for ultraviolet germicidal irradiation (UVGI), the REL is 0.006 joules per square centimeter (0.006 J/cm^2). The permissible irradiance for an 8 hour workday exposure is $\leq$0.2 $\mu W/cm^2$ or 6000 $\mu Wsec/cm^2$.

Each area where **Class 2** and **Class 3a lasers** are used shall be conspicuously posted with this standard **laser warning** sign.
Note: - Protective eyewear which absorbs the exact wave length of light produced by the laser in use shall be worn by all personnel with potential for exposure to this laser source or to the reflected beam.

All areas where **Class 3b and Class 4 lasers** are used shall be labeled with the **Danger - Laser Radiation sign.** Posting of this sign indicates an awareness of and use of all applicable administrative and engineering controls to prevent exposure.

Note: Protective eyewear which absorbs the wavelength of light produced by the laser shall be worn whenever the laser is activated.

This **Cancer Suspect Agent** label is posted when chemicals, which are classified as suspect human or animal carcinogens by the International Agency for Research on Cancer Monographs (IARC), National Toxicology Program (NTP) or by OSHA, are used or stored in the laboratory or work area.

The **carcinogen symbol** is posted when chemicals, classified as known human carcinogens by the National Toxicology Program (NTP), International Agency for Research on Cancer monographs (IARC) and OSHA, are used or stored in the laboratory or work area.

Rooms and areas used by multiple users or high volume users of chemicals for the storage of chemicals in excess of one (1) day's supply shall conspicuously post the area with the **Caution** - **Chemical Storage Area** sign.

Separate rooms, cabinets or areas where corrosives (concentrated acids or bases) in excess of one (1) day's supply are stored shall be conspicuously posted with the Caution **- Corrosive Materials sign.**

This **toxic chemicals** sign shall be posted in all areas or rooms where hazardous chemicals with an American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 5 ppm or less are used or stored. (A list of chemicals with a TLV ≤ 5 ppm can be found in the Johns Hopkins University Safety Policy and Procedures Manual)

This **toxic gas** sign shall be posted in all areas or rooms where toxic gases with an American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) of 10 ppm or less are used or stored. A partial list of these toxic gases and their TLV or ceiling level (C) is below:

Arsine (0.05 ppm)	Hydrogen sulfide (10 ppm)
Boron trichloride (1 ppm)	Methyl mercaptan(0.5 ppm)
Boron trifluoride (1 ppm C)	Monomethylamine(5 ppm)
Butadiene (10 ppm)	Nitrogen dioxide (3 ppm)
Chlorine (1 ppm)	Phosgene (0.1 ppm)
Cyanogen (10 ppm)	Phosphine (0.3 ppm)
Diborane (0.1 ppm)	Silane(5 ppm)
Dimethylamine (5 ppm)	Silicon tetrafluoride (0.1 ppm)
Hydrogen bromide (5 ppm)	Sulfur dioxide (2 ppm)
Hydrogen chloride (5 ppm C)	Sulfur tetrafluoride (0.1 ppm C)
Hydrogen fluoride (3 ppm C)	Trimethylamine (5 ppm)
Hydrogen selenide (0.05 ppm)	

All rooms and areas, accessible to personnel, containing, unguarded (exposed, open) electrical equipment **in excess of 600 volts** shall be conspicuously posted with the **Caution - High Voltage** sign.

All rooms or areas, accessible to personnel, with exposed (open) electrical systems or live parts **less than 600 volts** shall be labeled with the **Caution - Electrical Hazard** sign.

Identifies laboratories **which have one or more approved flammables storage cabinets**

This sign indicates that **eating, drinking, smoking, handling of contact lenses, and applying cosmetics are not permitted** in the posted work area because there is reasonable likelihood of exposure to hazardous chemicals, radioactive materials or potentially infectious materials.

This **eye protection required** label shall be posted in all areas, accessible to personnel, where there is a reasonable probability of

exposure to hazardous chemicals, potentially infectious agents or physical hazards which could result in injury that can be prevented by eye protection shall be conspicuously posted with this sign.

Each area or room, accessible to personnel, in which there is a potential for noise exposure which may equal or exceed an 8-hour time-weighted average sound level (TWA) of 85 dBA. (OSHA standard 1910.95) shall be posted with the **hearing protection required** label.

This **protective clothing required** sign shall be posted when work conditions require specific protective clothing which is beyond the standard laboratory coat. Personal protective equipment selected for this work area shall be based on an evaluation of the task specific conditions and the hazards and potential hazards that are encountered. In compliance with the Workplace Hazard Assessment - OSHA standard CFR 1910.132.

(Continued next page)

NFPA 704 Standard System for the Identification of the Fire Hazards of materials.

This system identifies the hazards of a material in terms of three principle categories. "Health" (blue), "Flammability" (red) and "Reactivity" (yellow). It indicates the degree of severity by a numerical rating that ranges from four (4), indicating severe hazard, to zero (0), indicating no hazard. (See below). The fourth space, at the six o'clock position (white) is reserved for indicating any unusual reactivity with water by the symbol ₩ but may also be used to indicate other unusual or special hazards but only if the ₩ symbol is not needed (examples -radioactive symbol, oxidizer (OX), corrosive (COR). Numbers and letters shall be printed in the appropriate colored diamond with a felt-tip, permanent ink marking pen.

CAUTION

BIOHAZARD
INFECTIOUS AGENTS
BIOSAFETY LEVEL 2

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 5 shall be posted on the doors of laboratories where work is performed with infectious agents that require special conditions for containment. The need for this sign will depend on the nature of the work as well as the pathogenicity of the agent.

Principal investigators and/or laboratory supervisors are responsible for assessing risk and determining the need for the warning signs subject to approval by the Biosafety Officer. All posted areas shall be registered with the Biosafety Officer.

Laboratories posted with the BSL 2 sticker are in compliance with the BMBL* requirements for safety equipment and facility design for this level of containment. Laboratory staff have been trained to use the work practices required at the BSL 2 level.

DAY: Visits in these areas are prohibited unless the visitor has permission from the investigator(s) in charge, who will be responsible for the safety of the visitor while he is in the area. Visitor access to the laboratory must comply with institutional policy.

Maintenance personnel should contact the laboratory supervisor for clearance before working in the area.

NIGHT: Normally, each laboratory shall be "secured" at the end of each work day. Infectious materials shall be stored in refrigerators, incubators, etc.; table tops shall be wiped down with an appropriate disinfectant; contaminated glassware and equipment shall be sterilized or contained in covered pans. All hazard sources shall have been contained for the night.

The area shall be safe for entry by night cleaning crews and other service personnel; however, these personnel must observe specific instructions and precautions and must not work on any equipment with a 5"x 5" **CAUTION - BIOHAZARD - RESTRICTED AREA** sign. (See Exhibit 17).

*CDC/NIH 1993 Biosafety in Microbiological and Biomedical Laboratories, 3rd Edition. U.S. Department of Health and Human Services, Public Health Service and subsequent revisions

Exhibit 5

CAUTION

BIOHAZARD
POTENTIALLY INFECTIOUS MATERIAL
BIOSAFETY LEVEL 2

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 6 shall be posted on doors of laboratories where work is being done with clinical specimens (sputum, blood or other body fluids, tissue specimens, etc.) or environmental samples presumed to contain infectious agents.

Principal investigators and/or laboratory supervisors are responsible for assessing the risk and determining the need for the warning signs subject to approval by the Biosafety Officer. All posted areas shall be registered with the Biosafety Officer.

Laboratories posted with the BSL 2 sticker are in compliance with the BMBL* requirements for safety equipment and facility design for this level of containment. Laboratory staff have been trained in the work practices required at the BSL 2 level.

The containment elements described above are consistent with the OSHA Standard 49CFR1910.1030 Occupational Exposure to Bloodborne Pathogens that requires the use of specific precautions for **all** clinical specimens and other potentially infectious material (Universal Precautions).

DAY: Visits in these areas are prohibited unless the visitor has permission from the supervisor or investigator in charge, who will be responsible for the safety of the visitor while he is in the area. Visitor access to the laboratory must be in compliance with institutional policy.

Maintenance personnel should contact the laboratory supervisor for clearance before working in the area.

NIGHT: Normally, each laboratory shall be "secured" at the end of each work day. Infectious materials shall be stored in refrigerators, incubators, etc.; table tops shall be wiped down with an appropriate disinfectant; contaminated glassware and equipment shall be sterilized or contained in covered pans. All hazard sources shall have been contained for the night.

The area shall be safe for entry by night crews and other service personnel; however, these personnel must observe specific instructions and precautions and must not work

on any equipment with a **CAUTION - BIOHAZARD - INFECTIOUS AGENTS - RESTRICTED AREA** sign. (See Exhibit 17)

Exhibit 6

CAUTION

BIOHAZARD
INFECTIOUS AGENTS
BIOSAFETY LEVEL 3
RESTRICTED AREA

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 7 shall be posted on doors of laboratories with infectious agents or materials requiring the special conditions of containment of Biosafety Level 3. Laboratories posted with the BSL 3 sticker are in compliance with the BMBL* requirements for safety equipment and facility design for this level of containment. Laboratory staff have been trained in the special work practices required at the BSL 3 level. A smaller sign (5"x 5"), indicating **CAUTION - BIOHAZARD - RESTRICTED AREA** shall be posted on incubators, refrigerators, cabinets, and other equipment for containment of infectious agents.

DAY: When the **RESTRICTED AREA** sign is posted for entire work areas, access is restricted to persons whose presence is required for program or support purposes. Routine cleaning of posted areas shall be done by laboratory personnel.

When the **CAUTION - BIOHAZARD - RESTRICTED AREA** sign is posted on laboratory equipment or on an entire work area, mechanical failures of equipment shall not be repaired until the hazardous agents have been removed; the equipment decontaminated, if required; and assurance given by the investigator(s) that the equipment can be safely repaired.

NIGHT: At the end of the working day the laboratory shall be "secured" as described in Exhibit 5 and 6. All hazard sources shall have been contained. Facilities maintenance and housekeeping procedures, restricted during the working day, may be performed at night by arrangement with the principal investigator or laboratory supervisor.

Emergency crews and fire personnel may respond to emergencies in the area. Equipment posted with a 5"x 5" **CAUTION - BIOHAZARD - RESTRICTED AREA** sign shall be left alone, except to disconnect external sources of electrical power, gas or water until the listed investigator(s) is present to determine the safety of further emergency procedures.

Failure to observe this sign may result in exposure to pathogenic agents and can also result in disciplinary action.

Exhibit 7

CAUTION

BIOHAZARD
INFECTED ANIMALS

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 8 shall be posted when animals are inoculated with biohazardous agents or materials by injection, exposure to aerosols, or by other means. The sign should be posted when animals are in quarantine and may shed infectious agents. This sign may also be posted when animals must not be exposed to any source of infection, including visitors.

Access to this area is restricted to persons whose presence is required for program or support services.

Animal care personnel must be informed of the potential hazards to which they may be exposed and provided with specific instructions for handling the infected animals.

Maintenance personnel and visitors to animal quarters posted with the **CAUTION-INFECTED ANIMALS** sign must be accompanied by personnel assigned to the area.

This **INFECTED ANIMALS** label may be accompanied by a **BIOSAFETY LEVEL 2** containment or a **BIOSAFETY LEVEL 3** containment label indicating that the facility, the safety equipment and the work practices used in the facility meet the criteria for vertebrate animal biosafety at the specified level. (BMBL*).

Exhibit 8

CAUTION

RADIOACTIVE MATERIALS

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 9 shall be posted on the doors of each area or room in which radioactive material is used or stored and which contain radioactive materials exceeding the permissible quantities as specified in the Standards for Protection Against Radiation, Atomic Energy Commission (10 CFR 20), and Maryland Regulations. The Radiation Safety Officer and/or the Joint Committee on Radiation Control will determine the need for the warning signs. All posted areas shall be registered with the Radiation Safety Officer.

The requirement for personnel monitoring equipment (film badges, pocket dosimeters, film rings, etc.) shall be determined by the Radiation Safety Officer, who will supply the monitoring equipment and maintain the records of individual exposure to radiation.

DAY: Visitors in these areas are prohibited unless the visitor has permission from the investigator, supervisor, or administrator in charge, who will be responsible for the safety of the visitor while he is in the area. Maintenance personnel and Housekeeping should contact the investigator or laboratory supervisor for clearance before working in the area. The requirement for personnel monitoring equipment for visitors, housekeepers, and maintenance personnel shall be consistent with the policy established for the area.

NIGHT: Normally, each laboratory shall be "secured" at the end of each work day. Radioactive materials shall be shielded, if necessary, and stored in properly posted cabinets, refrigerators, etc.; table tops and equipment shall be decontaminated; radioactive glassware and equipment for cleaning shall be in pans; and radioactive waste should be in labeled disposal cans or barrels.

The area shall be safe for entry by night cleaning crews and other service personnel; however, these personnel must observe specific instructions and precautions and must not work on any equipment with a 5"x 5" **CAUTION - RADIOACTIVE MATERIALS - RESTRICTED AREA** sign. (See Exhibit 17).

Exhibit 9

CAUTION

RADIATION AREA

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 10 shall be posted in any area, accessible to personnel, in which there exists radiation at such levels that a portion of the body could receive in any one hour a dose in excess of 5 millirems , or in any 5 consecutive days a dose in excess to 100 millirems.
Only persons who work in this area and patients may enter when this sign is posted. All personnel shall wear or carry personnel monitoring equipment.

DAY: During the working day, service personnel such as housekeepers, engineers, repair crews, etc. shall not enter unless accompanied by the investigator or supervisor in charge of the area. Mechanical failures in such an area will be left alone until the investigator(s) named on the sign have verified that the area can be safely entered.

NIGHT: At the end of the working day, the area shall be secured as described for Exhibit 9. All hazard sources shall have been contained. Housekeeping and facilities maintenance procedures, restricted during the working day, may be performed at night.

Emergency crews, and fire personnel may respond to fires and other emergencies in the area. Equipment posted with a 5"x 5" **CAUTION - RADIATION AREA - RESTRICTED AREA** sign (Exhibit17) shall be left alone, except to disconnect external sources of electrical power, gas or water until the investigator(s) named on the sign or the Radiation Safety Officer are present to determine the safety of further emergency procedures.

Failure to observe this sign may result in exposure to extremely hazardous radiation and can also result in disciplinary action.

These **CAUTION - RADIATION AREA** signs shall be removed when the hazard no longer exists.

Exhibit 10

CAUTION

HIGH RADIATION AREA

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 11 shall be posted in any area accessible to personnel, in which there exists radiation at such levels that a major portion of the body could receive in any one hour a dose in excess of 100 millirems .

Only persons who work in this area and patients may enter when this sign is posted. All personnel shall wear or carry personnel monitoring equipment.

DAY: During the working day, service personnel such as housekeepers, engineers, repair crews, etc., shall not enter unless accompanied by the investigator or supervisor in charge of the area. Mechanical failures in such an area will be left alone until the investigator(s) named on the sign have verified that the area can be safely entered.

NIGHT: At the end of the working day the area shall be "secured" as described for Exhibit 9. All hazard sources shall have been contained. Housekeeping and facilities maintenance procedures, restricted during the working day, may be performed at night.

Emergency crews and fire personnel may respond to fires and other emergencies in the area. Equipment posted with a 5"x5" CAUTION **- HIGH RADIATION AREA - RESTRICTED AREA** sign shall be left alone, except to disconnect external sources of electrical power, gas or water until the investigator(s) named on the sign or the Radiation Safety Officer are present to determine the safety of further emergency procedures.

Failure to observe this sign may result in exposure to extremely hazardous radiation and can result in disciplinary action.

CAUTION - HIGH RADIATION AREA - RESTRICICITED AREA signs shall be removed when the hazard no longer exists.

Exhibit 11

CAUTION

HAZARDOUS CHEMICAL
CANCER SUSPECT AGENT

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x10" sign illustrated in Exhibit 12 shall be posted at the entrance(s) to each area or room in which suspect carcinogens, regulated by the Occupational Safety and Health Administration (OSHA), are used or stored. These regulated suspect carcinogens are identified in the Occupational Safety and Health Standards, Subpart Z - Toxic and Hazardous Chemicals (29 CFR 1910.1000 ff).

Instructions shall be posted with this sign informing employees of the procedures that must be followed on entering and leaving the regulated area.

Visits in these areas are prohibited unless the visitor is accompanied by the supervisor or investigator in charge, who will be responsible for the safety of the visitor in the area. Access to visitors must be in compliance with institutional policy.

Within each regulated area, all containers of cancer suspect agents shall have warning words **"CANCER SUSPECT AGENT"** displayed immediately under or adjacent to the contents identification.

Maintenance personnel should contact the laboratory supervisor for clearance before working in the area.

Normally, each laboratory shall be “decontaminated” at the end of each day, weighing areas and bench tops shall be cleaned, contaminated absorbent pads shall be changed and properly disposed of, chemicals shall be in their proper storage areas, contaminated glassware and equipment shall be in pans; and hazardous waste shall be in appropriate containers.

The area shall be safe for entry by night cleaning crews and other service personnel, however, these personnel must observe specific instructions and precautions and must not work on any equipment labeled with a 5"x 5" **CAUTION - CANCER SUSPECT AGENT - RESTRICTED AREA** sign.

Principal investigators and/or laboratory supervisors are responsible for assessing the risk and determining the need for the warning signs subject to approval by the Environmental

Health Officer. All posted areas shall be registered with the Office of Safety and Environmental Health.

Exhibit 12

CAUTION

CANCER
HAZARD

ADMITTANCE TO AUTHORIZED PERSONNEL ONLY

The 10"x 10" sign illustrated in Exhibit 13 shall be posted at the entrance(s) to each designated areas in which the select carcinogens regulated by the Occupational Safety and Health Administration (OSHA) are used or stored. These known human carcinogens are identified in the Occupational Safety and Health Standards, Subpart Z - Toxic and Hazardous Chemicals (29 CFR 1910.1450) Occupational Exposure to Hazardous Chemicals in Laboratories.

Instructions shall be posted with this sign informing employees of the procedures that must be followed on entering and leaving the designated area.

Visits in these areas are prohibited unless the visitor is accompanied by the supervisor or investigator in charge, who will be responsible for the safety of the visitor in the area. Access to visitors must be in compliance with institutional policy.

Within each designated area, all containers of the regulated carcinogens shall have warning words **"CANCER - HAZARD"** or the **CANCER HAZARD symbol** displayed immediately under or adjacent to the contents identification.

Lists of human carcinogens as defined by OSHA, the National Toxicology Program (NTP) and the International Agency for Research on Carcinogens (IARC) monographs are available from the Office of Safety and Environmental Health.

Maintenance personnel should contact the laboratory supervisor for clearance before working in the area.

Normally, each laboratory shall be “decontaminated” at the end of each day, weighing areas and bench tops shall be cleaned, contaminated absorbent pads shall be changed and properly disposed of, chemicals shall be in their proper storage areas, contaminated glassware and equipment shall be in pans; and hazardous waste shall be in appropriate containers.

The area shall be safe for entry by night cleaning crews and other service personnel, however, these personnel must observe specific instructions and precautions and must not

work on any equipment labeled with a 5"x 5" **CAUTION - CANCER HAZARD - RESTRICTED AREA** sign.

Principal investigators and/or laboratory supervisors are responsible for registering all areas posted with this sign with the Office of Safety and Environmental Health

Exhibit 13

CAUTION

EYE PROTECTION
REQUIRED

The label illustrated in Exhibit 14 will be affixed to each 10" x 10" placard posted in each area, accessible to personnel, where there is a reasonable probability of exposure of the eye to chemical, biological, radiological, and physical hazards which could be prevented by eye or face protection

This requirement is in compliance with OSHA standards 29 CFR 1910.133- Eye and Face Protection; 29CFR1910.1030- Occupational exposure to Bloodborne Pathogens; 29CFR1910.1450- Occupational Exposure to Hazardous Chemicals in Laboratories; and the JHU Safety Policy 203-Use of Protective eye and Face equipment (Johns Hopkins University Safety policy and Procedure Manual, pp 206-208)

Eye Protection Rules

Glasses with side shields
- Minimum acceptable eye protection in posted areas
- Use when there is a splash hazard with small quantities of chemicals, e.g. opening a bottle or microcentrifuge tube.
- Use when need protection from impact with small particles.

Goggles
- Use when working with liquids that are highly caustic or with large volumes of hazardous chemicals, e.g. 1 liter or more.

Face Shield
- Use when working with large volumes of hazardous chemicals.
- When there is a need to protect eyes and face.
  When removing closed containers from liquid nitrogen or other cryogenic liquid.

Special Eye Protection
- Use an ultraviolet (UV)absorbing, full face shields when working with a transilluminator.
- Use wavelength specific protective eyewear when working in the area of Class 3b and Class 4 lasers.

Exhibit 14

DANGER

DO NOT ENTER
CONTAMINATED AREA!

The sign illustrated in Exhibit 15 will be posted only on a temporary basis when an extremely hazardous condition exists. It will not be posted on long-term hazardous areas.

If a spill has occurred and the area is contaminated or if it is not possible to "secure" the laboratory because of the work being done, this sign designating an unsafe condition will be taped on the door. (Normally each laboratory shall be "secured" at the end of each work day and the area will be safe for entry by housekeepers and other service personnel).

NO ONE SHALL ENTER THIS AREA WHILE THIS SIGN IS POSTED unless accompanied by investigator(s) named on the sign. The investigator shall be responsible for the safety of all personnel entering the area. The sign shall remain posted until the hazard is removed.

The Biosafety Officer, the Radiation Safety Officer, or the Environmental Health Officer shall be notified whenever this DANGER **- CONTAMINATED AREA** sign is posted. They shall be informed of the circumstances requiring the use of the sign and the area posted. A determination will be made on the procedures to be used to abate the hazardous condition and the personal protective equipment required. Usually the conditions which require this posting will also require the assistance of the Office of Safety and Environmental Health in the remediation or clean-up. The assisting OSEH staff will determine when the hazardous condition is abated and when the sign can be removed.

Exhibit 15

MULTIPLE HAZARDS

The signs in Exhibit 16 show the most frequently posted multiple hazard warnings for biomedical research and diagnostic laboratories. The larger (10" x 10") placards should be posted at accesses to the laboratories. The smaller (5" x 5") signs (Exhibit 17) should be affixed to incubators, freezers, refrigerators, cabinets and other equipment used for containment, incubation, storage, or decontamination of infectious agents, radioactive materials and/or hazardous chemicals.

Exhibit 18 illustrates a caution sign identifying five (5) hazards and three (3) workplace practice requirements. The hazard level of the placard is determined by the greatest hazard.

Exhibit 16

Exhibit 17

Exhibit 18

A

Acquired immunodeficiency syndrome (AIDS), 9, 10, 23, 27
  reporting exposures, 2, 13, 21
  see also Human immunodeficiency virus (HIV)
Aerosols and droplets
  containment/control equipment, 53, 202
  procedures generating from sonicators, homogenizers, and mixers, 57
Alcohol disinfection, 90
Animal pathogens, restricted, 35, 132
Animals, see Laboratory animals
Anthrax, 128
Arboviruses, 131
Autoclave, 69, 72

B

B-virus, 18, 129, 131
Bacillus anthracis, 128
Bacillus subtilis, 127
Biological safety cabinets,
  certification of, 257, 258, 262
  characterizes, 205
  Class I, 205, 219
  Class II, 206, 221, 223, 226, 228
  Class III, 206, 230
  cleaning, 213
  decontamination of, 210
  exhaust air, 248, 255
  HEPA filter, 218
  proper use of, 208, 210, 236
  purchasing, 237, 244
  testing and monitoring of, 108, 326
  thimble connection, 248
  vendors, 242
Biosafety principles
  animal biosafety levels, 35
  biosafety levels, 32, 33, 34, 35, 36, 38, 43
  facility design, 38
  information sources, 36

laboratory practice and technique, 21
Biosafety Level 1
criteria, 32, 140

Biosafety Level 2
criteria, 33, 141, 278, 280
Biosafety Level 3
containment requirements, 38
criteria, 7, 34, 143, 282
Blastomyces dermatidis, 128
Body fluids,
HBV exposure risk from, 9
spill cleanup, 69
universal precautions, 5
Botulism, 128
Brucella spp., 130

C

Campylobacter fetus jejuni, 128
Centers for Disease Control and Prevention
guidelines for biosafety, 36
universal precautions, 27
Chemicals, hazardous, 292, 294
Chlamydia psittaci, 128
Chlamydia trachomatis, 128
Cholera, 128
Clostridium botulinum, 128
Clostridium tetani, 128
Coccidioides immitis, 131
Containment,
approaches, 21, 208
Biosafety Level 32,
Biosafety Level 2, 33,
Biosafety Level 3, 34
biosafety principles, 23, 43
defined, 44, 202, 203, 208
experimental animals, 68
filters, 203

laboratory, 38, 59, 67
packaging for shipment, 62
pipetting devices, 47
on sonicators, homogenizers, and mixers, 57, 208
testing and certification of, 262
see also Biological safety cabinets,
Corynebacterium diphtheriae, 128

Cowpox, 129
Creutzfeldt-Jakob agent,
inactivation of, 91
Crypotcoccus neoformans, 128
Cytomegalovirus, 129

D

Decontamination
freezers, 61
of HEPA filters, 85
validation of, 73
of wastes for disposal, 25, 31, 49, 69
of work surfaces, 25
Diphtheria, 128
Disinfectants,
alcohols, 90
aldehydes, 90
chlorine compounds, 91
formalin, 90
glutaraldehyde, 90
iodophors, 89
mercurial, 91
phenolic, 88
for radioactive spills, 94
quaternary ammonium compounds, 88
in vacuum system, 59
for water baths, 58

E

Emergencies
damaged shipments, 62
decontamination, 31, 69
preparation and general procedures, 8, 42
spills and releases, 25, 93, 94, 298
Entamoeba histolytica, 129

Equipment
centrifuges and ultracentrifuges, 53
decontamination of, 72, 85, 88
pipetting devices, 47
sonicators, homogenizers, and mixers, 57
see also Biological safety cabinets; Containment
Escherichia coli, 126, 128
Ethylene oxide, 84
Eye protection, 296

F

Facilities
Biosafety Level 1, 32
Biosafety Level 2, 33, 278, 280
Biosafety Level 3, 34, 282
constructing, 38,
laboratory design, 38, 67
Formaldehyde, 85
Formalin, 90
Francisella tularensis, 130

G

Glutaraldehyde, 90
Gonorrhea, 128

H

Health care workers, 119
precautions to prevent HIV transmissions, 9, 23
Hemorrhagic fever, 131

HEPA filters
  biological safety cabinets, 203, 218
  decontamination of, 85
  ultracentrifuges, 54
  vacuum lines, 59

Hepatitis A virus, 129
Hepatits B virus (HBV), 9, 129
  body fluid sources, 23
  immunization against, 26
  protective clothing, 24
  recommended precautions, 23
  universal precautions, 23
Herpesviruses, 129
Herpesviruses simiae (B-virus), 18, 131
Histoplasma capsulatum, 131
HIV, see Human immunodeficiency virus,
Housekeeping,
  personnel safety measures, 25
  waste disposal, 49
Human immunodeficiency virus (HIV),
  animal biosafety level criteria, 24, 68
  biosafety level criteria, 23,
  body fluid sources, 23
  decontamination, 25, 69
  exposure management, 9,
  guidelines for handling, 27
  housekeeping precautions, 25
  inactivation of, 25
  laboratory facilities, 26, 67
  laboratory precautions, 23
  medical surveillance programs, 21, 26
  protective barriers, 24
  seroconversions in laboratory workers, 26
  spill cleanup/contamination, 25, 93
  agent summary statements, 27
  universal precautions, 23

in waste, 49
see also Health care workers

I

Immunization of workers,
recommendations, 8, 21, 23
see also Vaccinations/vaccines
Importation of biologicals, 62, 97

Inactivation
Creutzfeldt-Jakob agent, 91
of cultures, 69, 72
Human Immunodeficiency Virus, 25
Mycobacterium spp., 88
Q fever, 31
validation of, 73
see also Decontamination; Disinfectants
Infectious agents
bacterial, 4, 17, 128, 130
fungal, 128, 131
parasitic, 129
rickettsial, 12, 28, 129, 131
risk, 44
viral, 4, 18, 23, 45, 129, 130, 131, 132
see also specific agents
Influenza virus, 129

J

Junin virus, 131

K

Klebsiella, 128

L

Labeling
of cages, 29
and levels of laboratory practice, 21, 23
shipments of biological materials, 97
Laboratory animals,
Biosafety Levels, 35
containment equipment for, 202, 205
facility design for, 38, 284
safety procedures, 35
waste handling from, 31

Laboratory environment/design
access control, 26, 265
guidelines, 43
physical features, 38, 67
safety equipment, 202, 215
vacuum system, 59
ventilation, 40
voluntary code of practice, 21
waste handling, 49
see also Facilities
Laboratory practices
academic laboratories, 21, 46, 65, 202
Biosafety Level 1, 32
Biosafety Level 2, 24, 33, 278, 280
Biosafety Level 3, 34, 282
biosafety principles, 36
HIV handling precautions, 9, 23, 27, 91
standard operating procedures, 7
training of personnel, 1
universal precautions, 23
Laboratory waste
animal bedding material, 31, 68
body fluids, 23
decontamination of, 69, 72, 88
disposal, 49, 52
needles and other "sharps", 49
see also Waste handling and disposal

Lassa virus, 131
Legionella pneumophila, 128
Leishmania spp., 129
Leprosy, 128
Leptospira interrogans, 128
Lymphocytic choriomeningitis virus, 131
Lymphogranuloma venereum, 129

M

Marburg virus, 131
Measles virus, 129
Meningococcal meningitis, 128
Monkeypox, 129

Mycobacterium
  avium, 128
  bovis, 130
  inactivation of, 72, 88, 91
  tuberculosis, 130

N

Naegleria gruberi, 129
National Sanitation Foundation, 239, 263
Needles and other "sharps"
  disposal of, 49
  limiting use of, 24
Neisseria gonorrhoeae, 128
Neisseria meningitidis, 128
Nematode parasites, 129

O

Occupational infections
  assessment of risk, 5, 21
  highest risk organisms, 44, 45

see also specific infectious agents

P

Packaging of biological materials
  for disposal, 49
  needles and other "sharps", 51
  for shipment, 97
Parasitic agents, 129
Phenolic compounds, 88
Pipetting devices
  hazards of, 47
  safety aids, 47
Plague bacillus, 130
Polio virus, 129
Pox viruses, 129
Protective clothing, 21, 24, 30
Pseudomonas pseudomallei, 130
Pulmonary diseases, Mycobacterium spp., 130

Q

Q fever
  immunization, 29
  resistance to inactivation, 31
  route of infection, 28
  registration of work with, 28
  risk of infection from, 28

R

Rabies virus, 129
Radiation, 288, 290
Radioactive materials, 286
Radioactive material spill, 94
Reovirus, 129
Rickettsia, 131
Rift Valley fever virus, 132
Rubella virus, 129

S

Safety management
  administrative organization and responsibilities, 1
  information resources, 27, 35, 97, 216
Salmonella spp., 128
Schistosoma spp., 129
Semiliki Forest virus, 131
Shigella spp., 128
Shipment of biologicals, 97
Signage
  Biohazard, Infectious Agents, BL2, 278
  Biohazard, Potentially Infectious Material, BL2, 280
  Biohazard, Infectious Agents, BL3, 282
  Biohazard, Infected Animals, 284
  Radioactive Materials, 286
  Radiation Area, 288
  High Radiation Area, 290
  Hazardous Chemical, Cancer Suspect Agent, 292
  Cancer Hazard, 294
  Eye Protection Required, 296
  Danger, Do Not Enter, Contaminated Area, 298
  Multiple Hazards, 300

Smallpox, 132
Spills and releases
  in biological safety cabinets, 93
  outside biological safety cabinets, 93
  radioactive biologicals, 94
Spirochete spp., 128
Sterilization (Decontamination)
  autoclaving, 72
  biological safety cabinets, 85
  of waste, 69
  see also Disinfectants
Streptococcal spp., 128
Syphilis, 128

T

Tetanus, 128
Tissue samples
  disposal of, 49
Toxoplasma spp., 129
Training in biosafety
  academic setting, 21
  Biosafety Level 3, 34
  responsibility for, 1, 4, 77
  use of containment equipment, 216
Transportation
  importation and interstate shipment of biologicals, 97
Treponema pallidum, 128
Trypanosoma spp., 129
Tuberculosis, 130
Tularemia, 130
Typhoid fever, 128

U

U.S. Department of Agriculture, 97
U.S. Department of Transportation, 97
U.S. Postal Services, 97
U.S. Public Health Services
  regulation of import and transport of etiologic agents, 97

V

Vaccination/vaccines, prophylactic, 8, 21
Vaccinia virus, 8, 129
Varicella virus, 129
Venezuelan equine encephalitis, 131
Vesicular stomatitis virus, 129
Vibrio cholerae, 128
Vibrio parahaemolyticus, 128

Viral hepatitis

risk of infection from, 23, 27
see also Hepatitis A virus; Hepatitis B virus (HBV)

W

Waste handling and disposal
autoclave decontamination, 72
animal bedding material, 31
basic principles, 49, 52
chemical disinfectants, 88
containment/packaging, 50, 97
cultures, 52
HIV precautions, 9, 23
needles and other "sharps", 49, 51
personnel protection, 21
regulation of, 49
steam autoclaving, 72
validation of decontamination methods, 72

XYZ

Yaba virus, 130
Yellow fever, 129
Yersinia pestis, 128

\BIOSAFMAN 6/19/95