Risk Assessment of Synthetic Genomics: A Biosafety and Biosecurity Perspective

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Introduction

The ability to synthesize molecules found in living organisms is not new for scientists in the fields of biochemistry and molecular biology. However, the "synthetic biology" made possible by the genetic mapping of microorganisms, plants and animals, including the human genome, has taken this area of science into new and relatively uncharted territory. The focus here will be on "synthetic genomics" in which genetic information is synthesized using chemical components and the genomic DNA sequence of an organism. This is how investigators at the State University of New York in Stony Brook, using a published genetic sequence, synthesized a DNA version of poliovirus in 2002. Using an enzyme, reverse transcriptase, they converted the DNA to RNA and were able to grow the virus in a cell-free extract. Their synthesized poliovirus caused paralysis in animals (Cello et al., 2002). One of the authors, Eckard Wimmer, warned: "*The world had better be prepared. This shows you can re-create a virus from written information*."

From a biosafety and biosecurity perspective the synthesis of etiologic agents is of concern because of the potential to create completely new combinations or chimeric genomes with enhanced virulence, extended host range, and resistance to antimicrobials, antivirals or vaccines. A major concern is that an agent which has been eradicated as a source of infectious disease, such as smallpox, and one which is in the process of being eradicated, such as poliovirus, will never be truly eliminated because the information for their synthesis is readily available in sequence databases.

The potential benefits of synthetic genomics include but are not limited to new sources of fuel, food, therapeutics and environmental remediation. Plans to utilize synthetic

genomics to obtain specific products or outcomes are well underway. A bacteriophage genome was synthesized by the Institute of Biological Energy Alternatives (Smith et al, 2003). They now plan to synthesize larger microorganisms, including a mycoplasma with a "minimal genome" (Hutchinson et al, 1999). Such an approach could be useful in beginning to address alternative biologically-based fuel sources, and other applications. Additionally, through rearranging genes, called "gene shuffling", and repeatedly selecting for specific traits, an organism can be made to make more of a desired product that it already produces. As explained by Maxygen: "The parents are a series of related genes. These are cut into pieces, shuffled together and then assembled to form a new genetic generation. Some of these daughter genes can manufacture proteins that are much better at certain tasks than nature's originals. The best ones can be screened out and shuffled to produce whole lineages of superior descendants, in a process mimicking evolution by natural selection" (Crameri et al., 1998).

The risks associated with the synthesis of these genomes could have biological, chemical and physical components. Unknown and thus unquantifiable risks associated with new organisms or products from this technology could include various levels of harm to humans, animals, plants, other microorganisms and the environment in the event of an unplanned release. This is not unlike the risks perceived to lurk in recombinant DNA research in the early '70s or the risks potentially associated with the return of the first lunar astronauts and the recent samples from Mars. For example, in an attempt to protect against the introduction of unknown organisms or materials from space, the National Aeronautics and Space Administration (NASA) developed the Lunar Receiving Laboratory where astronauts were to be quarantined. Built from plans developed at Fort Detrick in Maryland, it included ethylene oxide chambers for sterilization. Such situations, when approached with a rational, scientific risk assessment of the known and unknown factors, can result in appropriate recommendations for biosafety as well as biosecurity. The guidelines and regulations currently in use in these areas can and do encompass synthetic genomics.

It may be useful to start with definitions for relevant terms to assist in understanding precisely the scope of possible safety concerns that could be triggered by a widespread introduction of synthetic genomics technologies (or by any new technology):

Laboratory biosafety "is used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release" (Chapter 9, page 47, WHO, 2004). Biosafety for larger scale and industrial work also focuses on providing a safe environment for work with biohazardous agents and materials (Cipriano, 2000:2002; NIH, 2002, Appendix K).

The definition given in Biosafety in Microbiological and Biomedical Laboratories is similar: **Biosafety:** Development and implementation of administrative policies, work practices, facility design, and safety equipment to prevent transmission of biologic agents to workers, other persons, and the environment (CDC/NIH, 1999). (It should be noted here that the term "biosafety" may not be appropriate for use in protection of the integrity of the "species", as in the 2003 Cartagena protocol of the Conference of the Parties to the Convention on Biological Diversity. "Species integrity" "species purity" or even "species safety" would better define the actual use of the term and prevent the confusion which currently exists.)

Laboratory biosecurity "refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion or intentional release of pathogens or toxins. Effective biosafety practices are the very foundation of laboratory biosecurity activities" (Chapter 9, page 47, WHO, 2004.)

The definition given in BMBL, although similar, is focused on select agents: **Biosecurity:** Protection of high-consequence microbial agents and toxins, or critical relevant information, against theft or diversion by those who intend to pursue intentional misuse. (See also Appendix 1, this document.)

1. Biosafety in research laboratories in the US

An overview of control of biohazards shows that the information for personal and community protection has been available for over forty years, but it has not necessarily been used for training at sites of use nor has it been incorporated into many training curricula.

1.1 EARLY HISTORY OF LABORATORY BIOSAFETY IN THE USA. In classified work done during the 1940s to 1969 at Fort Detrick, MD, and other U.S. Army Chemical Corps installations, there was an urgent need to focus on protection of the worker as well as the community from agents of biological warfare.[1] The work was so secret that the advances in protection were not well known outside the field of experts who worked there and who met in closed conferences. Information was shared in the first Biological Safety Conference held at Ft. Detrick in 1955. By 1966, the conference had grown to include universities, private laboratories, hospitals, industrial complexes and 17 government installations with presentations no longer bound by security. At that 11th conference, Dr. Arnold G. Wedum, now known as the "Father of Biosafety" discussed the revised edition of *Assessment of Risk of Human Infection in the Microbiological Laboratory* in which he provided four indicators of risk to serve as guidelines for the safe handling of microorganisms:

- number of laboratory infections observed
- infectious human dose
- infection of uninoculated control animals caged with or near inoculated cagemates
- presence of microorganisms in urine and/or feces of inoculated animal.

[1] Much of the early history of Ft. Detrick can be found at http://www.detrick.army.mil/cutting_edge/index.cfm?chapter=titlepage. The historical content of the early meetings and resultant papers published has been compiled by Manny Barbeito and Dick Kruse and published in the Journal of the American Biological Safety Association, now called "Applied Biosafety." This history of these conferences is also available on the ABSA website www.ABSA.org.

The published edition included 530 references and assumptions for 130 organisms or diseases. Charles Baldwin of Dow Chemical described the inception of the red-on-yellow biohazard symbol, which was first displayed at this conference and which a professional opinion group selected as the symbol they deemed unique, with easily recognized distinctive colors (Barbeito and Kruse, 1997).[2]

After President Nixon ended the biological warfare research program in 1969 and with the advent of the recombinant DNA guidelines in 1976 and the related NIH-sponsored training of biological safety officers in the early 1980s, these pioneers welcomed many newcomers to the field of biosafety. The 49th Biological Safety Conference is scheduled to be held in Boston in October of this year (2006). The American Biological Safety Association was formed in 1984.

1.2 CLASSIFICATION OF ETIOLOGIC AGENTS ON THE BASIS OF HAZARD: THE FIRST WRITTEN GUIDELINES. In 1969, the Public Health Service and the USDA, as part of their "regulatory responsibility for quarantine and interstate shipment of etiologic agents", worked together to produce the first edition of the "Classification of Etiologic Agents on the Basis of Hazard", the first documented guidelines for work with infectious agents. They noted in their introduction: "This document provides a standard for evaluating the hazards associated with various etiological agents and defines minimal safety conditions for their management without restricting or hampering bona fide microbiological investigations." This small booklet included lists of agents in four categories of increasing risk to the healthy adult worker and gave the basis for the agent classifications, as well as descriptions of the level of competency and the containment requirements. (See Appendix 2, this document.) A fifth category, known as Class 5, consisted of animal agents excluded from the USA by law (Foot-and-Mouth-Disease virus) and a list of agents excluded by USDA administrative policy

[2] For a firsthand account of the development of the symbol, and a graphic of it, see http://www.hms.harvard.edu/orsp/coms/BiosafetyResources/History-of-Biohazard-Symbol.htm

It should be noted that the number of academic departments of microbiology is shrinking every year, and very little if any hands-on training in good microbiological practices, including aseptic techniques, occurs outside these departments; sometimes, it does not even occur within these departments. In newer documents physical containment, given in terms of four Biosafety Levels, has become the focus of the recommendations and is given in agent summary statements in the guidelines from the Department of Health and Human Services (CDC/NIH, 1999).

The 4th edition of Classification of Etiologic Agents on the Basis of Hazard, published in 1974 and reprinted through 1976, continued to offer very concise guidelines for the general safety in handling of microorganisms: "The best way to maintain laboratory safety is to practice correct and careful laboratory techniques, including effective decontamination and sterilization procedures, at all times. The laboratory's isolation and containment requirements are to supplement, not to supplant, good laboratory practices and sound scientific judgment. However, in an adequately isolated and properly equipped laboratory with correctly directed airflow, a scientifically and technically competent investigator can confidently work even with the most hazardous agents, provided the safety cabinets are selected to meet the requirements of the work. Of the several available cabinet types, the investigator should select the one which meets requirements for the maximum risk he expects to encounter." The CDC's Office of Biosafety (now called Health and Safety) was available for consultation on the handling of etiologic agents.

The CDC list of the four classes of human pathogens and the USDA restricted agents was widely disseminated and continued to be used, for example, in an Appendix of the NIH guidelines, well into the 90's, long after it had been replaced in 1984 by the first edition of the CDC/NIH "Biosafety in Microbiological and Biomedical Laboratories." The World Health Organization, the European Union, Canada, Australia and New Zealand built on the older

agent classification model to provide definitions for four Risk Groups (RG) of agents. (Appendix 3, this document)

1.3 BIOSAFETY IN MICROBIOLOGICAL AND BIOMEDICAL LABORATORIES (BMBL). The information given in the BMBL included a new format of agent summary statements to assist in the selection of the appropriate containment for diagnostic clinical work, research and animal studies. More detailed recommendations for personal practices, safety equipment and facility design were given for each of the four biosafety levels of containment along with a separate set of four animal biosafety levels, due to the unique hazards associated with work in animals. The BMBL did not retain the list of etiologic agents based on hazard assessment, due to an unfavorable response from microbiologists who were concerned about costs and restrictions. The new format put the responsibility for risk assessment on the principal investigator or laboratory director and provided a limited number of agent summary statements for pathogens which have caused laboratory acquired infections (LAI) or could be of significant risk to the laboratory worker. Every known microorganism, and especially new or re-emerging pathogens, could not be addressed in BMBL. The mechanism for publication of timely information not covered by the current edition is to publish on the CDC website and in Morbidity and Mortality Weekly Report (MMWR) as well as professional journals. The current 4th edition is available online at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm Revised every 5 years, the 5th edition of BMBL is expected in 2006. The BMBL guidelines are considered the state of the art for the handling of infectious and toxic etiological agents of human disease in the USA. Section V on Risk Assessment can be found in Appendix 4 of this document.

1.4 NIH GUIDELINES FOR RESEARCH WITH RECOMBINANT DNA MOLECULES. With the advent of recombinant DNA research, and the Asilomar conference of potentially self-governing researchers in the mid 70s, guidelines were written for the biological and physical containment of such work. The first recombinant

DNA guidelines were published in 1976, and a series of technical advisory bulletins followed. The Office of the Director, NIH, whose signature was required for approval of potentially problematic experiments, also appointed a committee of experts, known as the Recombinant DNA Advisory Committee (RAC), to review and recommend revision, rejection or containment precautions for the work.

Institutional Biosafety Committees, formed to provide local oversight and approval, were provided with some training by the NIH for IBC representatives. An NIH sponsored train-the-trainer course was prepared by Dr. Donald Vesley with outlines and slides made available though the National Audiovisual Center as "Introduction to Biohazard Control". This material was to be used by local biological safety officers or other experienced professionals for training research workers (Appendix F of NRC, 1989). The World Health Organization Special Programme on Safety Measures in Microbiology sponsored the first WHO Global biosafety train-the-trainer course "Laboratory Biosafety Principles and Practices: An Instructor's Guide for Biosafety Training" in 1983 which included much of this material. There has been a recent outreach from NIH's Office of Biotechnology Activities (OBA) to provide updated training for IBC members throughout the country. The slides are available on the OBA website.[3] The current focus of the training is on the prescriptive requirements for compliance with the NIH guidelines, and not on the procedure-related training of those who do the hands-on work.

Over time, with an apparent lack of true hazards associated with the process of recombinant DNA, the guidelines were revised and relaxed. Most approvals were done locally by IBCs, although certain experiments were still to be approved by the RAC and NIH Director. Laboratory infections that have been reported were not related to the recombinant procedures and could have been prevented by using the biosafety guidelines and practices recommended for work with the infectious agent involved. For example, a vaccinia eye infection

[3] http://www4.od.nih.gov/oba/IBC/IBCindexpg.htm

and a skin infection occurred in workers who refused the recommended vaccine. Eye protection in one case and gloves in the second could have provided an appropriate barrier (Lewis et al., 2006; Mempel et al., 2003). Risk assessment from Section II of the NIH guidelines and the requirements for research scale (Appendix G) and large scale (Appendix K) work are provided in Appendix 5 of this document.

That the NIH guidelines can apply to synthetic genomics is seen from *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.[4]

Although the scope of the NIH guidelines clearly includes the biosafety of synthetic genomic work at laboratory and at large scale, the biosecurity issue has not been addressed in these guidelines. The NRC report *Biotechnology Research in an Age of Bioterrorism* (the Fink Committee report) begins to address some of these issues through its Recommendation #2 (establishment of a review system for "experiments of concern."). If the RAC or a similar body is to review experiments of concern in synthetic genomics, they and the local IBCs

[4] (http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html)

can utilize the information provided by the WHO and in the BMBL. Training programs available on the web can be modified for specific local use.

1.5 BIOSECURITY. Following the anthrax dissemination and deaths in the USA in 2001, regulations were promulgated to restrict the use of certain select agents. The CDC added a Select Agent Program with biosecurity as a major component. The Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (the Act) required institutions to notify the US Department of Health and Human Services (DHHS) or the US Department of Agriculture (USDA) of the possession of specific pathogens or toxins (i.e., select agents), as defined by DHHS, or certain animal and plant pathogens or toxins (i.e., high-consequence pathogens), as defined by USDA. Details can be found in the regulations:

- CDC and OIG, 2005; http://www.cdc.gov/od/sap/;
- APHIS,USDA,2005; http://www.aphis.usda.gov/programs/ag_selectagent/index.html
- Appendix F of BMBL, the 5th edition of which is to include further guidance on biosecurity policies and procedures, such as:

  - risk and threat assessment;
  - facility security plans;
  - physical security;
  - data and electronic technology systems;
  - security policies for personnel;
  - policies regarding accessing the laboratory and animal areas;
  - specimen accountability;
  - receipt of agents into the laboratory;
  - transfer or shipping of select agents from the laboratory;
  - emergency response plans; and
  - reporting of incidents, unintentional injuries, and security breaches

The National Science Advisory Board on Biosecurity (NSABB) is expected to offer more insight on requirements in this area: "The NSABB has been established to provide advice to federal departments and agencies on ways to minimize the possibility that knowledge and technologies emanating from vitally important biological research will be misused to threaten public health or national security. The NSABB is a critical component of a set of federal initiatives to promote biosecurity in life science research.

The NSABB is charged specifically with guiding the development of:

- A system of institutional and federal research review that allows for fulfillment of important research objectives while addressing national security concerns;
- Guidelines for the identification and conduct of research that may require special attention and security surveillance;
- Professional codes of conduct for scientists and laboratory workers that can be adopted by professional organizations and institutions engaged in life science research; and
- Materials and resources to educate the research community about effective biosecurity.

The NSABB is chartered to have up to 25 voting members with a broad range of expertise in molecular biology, microbiology, infectious diseases, biosafety, public health, veterinary medicine, plant health, national security, biodefense, law enforcement, scientific publishing, and related field. The NSABB also includes nonvoting ex officio members from 15 federal agencies and departments." (http://www.biosecurityboard.gov/)

A sample biosecurity training program developed by the Veteran's Administration can be found at
http://www1.va.gov/resdev/programs/biosafety/default.cfm#

1.6 OSHA'S BLOODBORNE PATHOGEN STANDARD. Finalized in 1992, this was the first regulation in which the Occupational Safety and Health Administration became involved with etiologic agents and infectious diseases to ensure a safe workplace for those potentially exposed to human blood and certain body materials as well as the viruses of hepatitis B and AIDS. The regulation was the result of petitions by the Service Employees International Union, a union of many frontline healthcare workers. The regulation has had a major impact on clinical laboratories and hospitals as well as on research with the agents covered. Concern about unsafe equipment led to further regulations requiring review of needle-stick and sharps injuries and selection of appropriate safety devices (OHSA, 2001). OSHA also monitors other infectious agents and materials under the general duty clause, the requirement for employers to provide a workplace free of recognized hazards. The OSHA website now contains fact sheets about infectious agents of concern in the workplace.

1.7 USDA'S SELECT AGENTS AND RESTRICTED (CLASS 5) AGENTS. 5) USDA'S SELECT AGENTS AND RESTRICTED (CLASS 5) AGENTS. The USDA has a list of agents that require permits for use some of which are handled under biosafety level 3 (BSL-3) or ABSL 3 (for animal containment) and even BSL 3 enhanced (BSL-3 with additional modifications, but clearly not a BSL-4 facility). Some specific containment conditions, BSL-3-Ag are applied when large animals cannot be caged and the room becomes the containment barrier. Use of such animals with eleven infectious agents requires BSL-3 Ag (Heckert and Kozlovac, 2006). (See below for detailed description of various containment facilities, and see Appendix 6, this document.)

1.8 OVERVIEW OF PREVIOUS FAILURES OF BIOLOGICAL CONTAINMENT. Workers assume the most risk in research enterprises including pathogenic agents. Most of the laboratory-acquired infections reported involved the person working with the agent, many of whom did not recall an accident (Harding and Byers, 2000).

A few accidents did involve some spread to co-workers (see Appendices 7 and 8, this document). Animals naturally infected with *Coxiella burnetii* in a San Francisco research facility were the source of infections in children and nurses who visited an area which should have been off-limits. According to the CDC web site: "Q fever outbreaks have resulted mainly from occupational exposure involving veterinarians, meat processing plant workers, sheep and dairy workers, livestock farmers, and researchers at facilities housing sheep. Prevention and control efforts should be directed primarily toward these groups and environments."

Community and other external populations are rarely involved. One exception was the release of anthrax in a 1979 industrial accident in Sverdlovsk, Russia. Finding the actual cause took many years of investigation (Miselson et al, 1994) and it was determined finally as the failure by maintenance personnel to replace a critical filter in a vent serving the anthrax production facility. The accidental smallpox release in a laboratory in Birmingham, England, resulted in two deaths but did not cause a community epidemic.

Release into community or environment has not been reported from US labs at Ft. Detrick, CDC or USDA (Cutting Edge. The History of Ft. Detrick, 4th ed. Oct, 2000 http://www.detrick.army.mil/cutting_edge/index.cfm?chapter=titlepage). There are sporadic cases of internal laboratory releases which infect workers and less frequently co-workers (see LAI, Collins, 1983, Harding and Byers, 2000, 2006 and Appendix 7, this document).

2. Concept of risk assessment

"*If reasonable precautions are to be taken against laboratory-acquired infections it is necessary to assess realistically the hazards that might be imposed on the laboratory worker and on the community during and as a result of work with any particular micro-organisms. It is a waste of time and resources to take elaborate precautions*

when the risks are negligible but foolish to take none if they are considerable. The precautions should be appropriate to the organism being investigated and the techniques used." (Collins, 1983, pg 53)

2.1 *Comprehensive risk assessment involves evaluating the agent-host-activity triad*

- Agent factors: Information on synthetic genomic constructs must come from the genetic sequence to be used and/or the sources of the genetic material to be combined. There is information on "wild type" or "type strains" of the pathogens in resources such as Bergey's Manual; ATCC catalogue; medical microbiology texts, BMBL agent summary statements, etc.)
- "Host factors" of lack of training in microbiology, recombinant DNA techniques, and specific techniques for synthetic genomics, and lack of competency in these techniques need to be addressed. This failure to train will not be resolved by promulgating regulations restricting the use of synthetic genomic processes. There are also other factors such as impaired immunity to be addressed.

2.2 Concept of chain of infection, which if broken reduces the risk and prevents disease

- Agent must be able to cause illness (pathogenic virus, toxigenic bacteria, etc.). For example, if the agent is inactivated or attenuated to a lesser degree of virulence it will not usually cause disease, even if it can still infect, but verification is needed.
- Reservoir: Agent must have a place to survive or replicate (intermediate host or reservoir). Example: Drain standing pools of water to prevent the breeding of an insect vector; kill the snail intermediate host of a parasitic disease.
- Exit point: Agent must be able to exit from the reservoir or host. Example: use algacide in cooling towers to kill algae and thus prevent amplification

and aerosol release of *Legionella;* send high temperature or flush with bleach to remove from water supply in hospital, thus preventing release from reservoir.

- Means of spread: mode of transmission. Direct (ingestion via mouth pipetting, injection with a contaminated needle or inoculation via animal bite) or indirect contact with agent such as contaminants on surfaces and particles in aerosols.
- Washing hands to remove transient contaminants and prevents many infections.
- Entry site: Agent must have a way to enter the host (route of entry): percutaneous (injection), ingestion, inhalation or contact with mucous membranes
- Susceptible host: the unimmunized or the immunologically impaired by disease or extremes of age (very young and very old). Note: immunization can be overwhelmed by a large dose of infectious agent; it changes the level of susceptibility.

The spread of infectious diseases at work can be stopped by breaking the chain:

- Killing the agent or replacing it with a non-pathogen
- Changing the environment so that the agent cannot survive
- Removing the agent's means of spread (mode of transmission)
- Making sure workers are immune to the agent and/or have protective equipment.
- Properly training workers on work practices to prevent illness.

2.3 Risk groups (WHO, NIH, EU, Canada, Australia, New Zealand)

The Risk Groups (RG) are based on:

- Severity of disease
- Individual and community risk (low to high)
- Host range (restricted or broad)

- Availability of treatment or prophylaxis (antibiotics, vaccines, etc)
- Endemicity (already present in the environment?)

2.4 Biosafety levels (CDC/NIH, 1999) used for risk management are based on

- The agent factors (see agent summary statements)
- The work to be done (clinical, research, large scale)
- The worker (host) factors (training, health, immunity)

Risk Assessment resources include:

- Chapter 5, BMBL: http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s5.htm
- Section II and appendix B, NIH guidelines http://www4.od.nih.gov/oba/rac/guidelines_02/NIH_Guidelines_Apr_02.htm#_Toc72615z http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm (See Appendix 4, this document).

Also see Appendix 8, this document, for a risk assessment matrix for agent hazards, and Appendix 9 for protocol hazards (Appendix 10 documents concentration and particle size of aerosols created during laboratory techniques). Finally, Appendix 11 is a risk assessment matrix for susceptibility to disease.

3. Selection of appropriate level of containment

(See NIH guidelines, Section II and CDC/NIH guidelines, BMBL, 4th ed)

It is the responsibility of the principal investigator or laboratory director to select the appropriate containment based upon their risk assessment. The institutional biosafety officer can provide assistance. Final local approval would come from the IBC or local biosafety committee, including the IRB (human subjects) and the IACUC (animal work) if appropriate. Higher level approvals from the RAC under NIH/OBA (or perhaps, at some point, the new NSABB) may be required. Select or restricted agents

are regulated by CDC or USDA, in which case that agency sets compliance and containment requirements. A Responsible Official (RO) and Assistant Responsible Official (ARO) represent the facility to ensure compliance with containment requirements.

3.1 Technologies for containment (See BSLs and ABSLs in BMBL and NIH guidelines Appendices G and K)

3.1.1 Administrative controls include standard operating procedures for:

- Housekeeping, spill clean up, decontamination, disinfection, sterilization and waste handling, packaging and disposal
- Hand washing, personal protective equipment, cleaning of uniforms etc.
- Reporting incidents, illnesses, accidents and injuries
- Medical program; vaccinations
- Training requirements and documentation
- Effective and safe use of equipment (biological safety cabinets, centrifuge, autoclave); equipment certification and validation requirements and records
- Limiting the number of workers exposed (access control)
- Monitoring and auditing checklists and procedures
- Avoiding exposure to infectious agents using the following practices at all times:
    - Do not mouth pipette
    - Manipulate infectious fluids carefully to avoid spills and the production of aerosols and droplets
    - Restrict the use of needles and syringes to those procedures for which there are no alternatives
    - Use needles, syringes and other “sharps” carefully to avoid self-inoculation
    - Dispose of “sharps” in leak and puncture resistant container

- Use protective laboratory coats and gloves
- Wash hands following all laboratory activities, following the removal of gloves, and immediately following contact with infectious materials
- Decontaminate work surfaces before and after use, and immediately after spills
- Do not eat, drink, store foods or smoke in the laboratory (NRC, 1989)

3.1.2 Engineering Controls

- General ventilation: maintaining a building at the proper temperature and humidity
- Local ventilation: such as isolation rooms, laboratory hoods, biological safety cabinets, and other means to control infectious agents
- Using safe needles and sharp devices designed to reduce the risk of needle sticks or other skin punctures and using puncture-proof sharps disposal containers
- Autoclaving or other sterilization methods

3.1.3 Primary barriers (personal protective equipment, safety cabinets,, other safety equipment, etc)

Personal protective equipment (PPE) includes:

- Respirators
- Gloves
- Face shields and eye protection
- Gowns, scrubs, head covers, booties, boots and other protective clothing

3.1.4 Secondary barriers: the physical facility

- If the facility is inappropriate for the proposed work, either the facility, the work or the method proposed for doing the work should be modified (NRC, 1989). BSL 1 and 2 do not require containment facilities
- BSL 3 and 4 are containment and high/maximum containment facilities respectively with specific features as described in BMBL
- BSL 3 Ag requires a containment facility that holds pressure and is used for loose, usually large animals (cattle, ostriches, etc) for work with 11 agents of concern (Heckert and Kozlovac, 2006)

4. Oversight mechanisms (See also Section 3)

A number of actors and agencies are responsible for ensuring that appropriate rules and guidelines are followed. These include, but are not necessarily limited to, the following:

Institutional: the principal investigator, the institutional biosafety officer, the institute's biosafety committee, IACUC auditing, certifications and commissionings.

Agency: CDC (import and select agents), EPA (TSCA and FIFRA rules), FDA (drugs, vaccines, and devices), USDA (APHIS, import and interstate movement, and select agents), DOT (transportation), Department of Commerce (export rules).

These and other cognizant bodies are reviewed at http://www.absa.org/resrules.html.

5. International considerations of interest

Because research is an international endeavor, it is critical to also understand rules and guidelines as asserted in other countries, not just in the United States, and not only by overarching bodies such as the World Health Organization. It seems very unlikely that there could ever be true harmonization of biosafety regulations, both for scientific reasons (the endemicity of microbes varies from locale to locale) and for cultural reasons. However, it will be worth considering if there could be any useful

global guidelines for biosafety with respect to synthetic genomics particularly (see Section VII).

See resource list for the WHO manual, regulations in Canada, EU, AU/NZ and some stringent country regulations, e.g., by competent authorities in UK. See also the commissioned paper on the oversight of biosafety in other countries by Franco Furger.

6. Special considerations for synthetic genomics

6.1 Any new safety issues?

It is difficult to foresee problems over and above what was expected with the early recombinant DNA experiments. As in the past, we will not know until they occur. However, it is worth reconsidering generally the types of problems or failures that have or could occur; in some cases these are directly relevant to synthetic genomics.

The problems could include:

- *Problem of unforeseen results*, particularly if the result is an unexpected increase in pathogenicity or virulence. A recent example of mice immunized against mousepox or naturally resistant mice that were nonetheless susceptible to a mousepox virus that had been modified by the addition of the interleukin IL-4. The purpose of the experiment was to create a mouse contraceptive; the outcome was clearly not what was expected. While the problem of unforeseen results is not unique to synthetic genomics, the combining of multiple sources of DNA sequence (not just, say, a bacterial vector and a specific gene as is exemplified by standard recombinant DNA techniques), particularly when this can occur very rapidly, may be of some concern.

- Broader host range than wild type. Synthetic genomics techniques might make quite simple, for example, the humanizing of zoonotic pathogens.
- Sheer volume of work that can be done. Using chip-based technologies, thousands or tens of thousand of "experiments" can potentially be done at one time. Although most of the synthetic genomics work occurring now is still at the "art" or "craft" level, it might be worth anticipating biosafety concerns now.

6.2 Lack of training in microbiology and/or recombinant DNA is problematic

Whether or not synthetic genomics is unique as a biotechnological tool, it is within the realm of technologies that rely heavily on good basic microbiological techniques. Although, as discussed above, the teaching of good microbiological techniques has faded somewhat over time in all departments, there is at least some tacit knowledge that is passed on and a good bit of structured training that still does take place in most biologically-oriented departments. Where synthetic genomics (and synthetic biology more generally) might be unique is the possibility that if the field does expand rapidly, as many are predicting, there could be an infusion of workers to the field who have literally no background in biology, let alone in microbiology. These could be people coming from engineering or physics background, and may never have stepped into a biology lab before they go about conducting their first experiment. If this is the case, the concern would then be about the general sorts of failures that can occur as a result of the use of poor technique:

- Failure to use aseptic technique and good microbiological practices can contaminate work or infect workers (SARS infections).
- Failure to understand routes of disease transmission can result in laboratory-acquired infections.
- Workers exposing co-workers, family or community in addition to themselves.

Further, not just for these new entrants to the field, but for many biologists, training has not included biosecurity aspects. For both biosafety and biosecurity, it is worth considering structured programs to train and mentor new investigators in synthetic genomics in good microbiological principles and practices, including specific procedures. NIH in fact developed in the 1980s an audiovisual program for non-microbiologists working with DNA (see Appendix F, NRC, 1989). The use of these structured programs, and a recommitment on the part of the community to training, may need to be considered as part of any research done in synthetic genomics.

6.3 Authority responsible for selection of containment (See also Section IV)

The same authorities that are responsible for oversight of biosafety are likely to be involved in the oversight of safety in synthetic genomics experiments and applications. The first point of contact in the chain is the principal investigator; for now, this is the person initially responsible for risk assessment. At the institutional level, the biosafety officer, the institutional biosafety committee, and the institutional animal care and use committee, if relevant, would all have some say.

At the national level, it remains to be seen to some degree how synthetic genomics is considered. Certainly, the NSABB (and thus NIH's Office of Biotechnology Activities) has taken an interest in synthetic genomics specifically. Both CDC and USDA will have an interest in synthetic genomics, particularly on the applications end as synthetic constructs begin to be used outside the laboratory.

Internationally, there has so far been little specific notice of synthetic genomics by the relevant offices that oversee biotechnology (including GMOs) in most of Europe. This is slowly changing, and whether the respective countries treat synthetic genomes as GMOs, as a generic biotechnology application, or in some

other way is not yet determined, and will be the focus of some discussion at the workshop.

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Biosafety Resource List

Guidelines, Manuals, Data Sheets

- Canadian Laboratory Biosafety Guidelines, 3rd Edition, 2004. Public Health Agency of Canada http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/index.html
- Canadian Material Safety Data Sheets (MSDS) for microorganisms. Health Canada, Office of Laboratory Security. http://www.phac-aspc.gc.ca/msds-ftss/index.html#menu
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Organization and Other Government Websites

- American Society for Microbiology (ASM) – http://www.asm.org
- American Biological Safety Association (ABSA) – http://www.absa.org
- American Type Culture Collection (ATCC) – http://www.atcc.org *(Check catalogs for detailed information and ATCC containment recommendations for cells and microorganisms.)*
- Department of Agriculture, Animal and Plant Health Inspection Service Plant virus lists and noxious weeds – www.aphis.usda.gov/ppq Veterinary Services – http://www.aphis.usda.gov/vs/ncie/ *(Information to import or to domestically transfer etiologic agents of livestock, poultry & other animals or materials that might contain these etiologic agents.)*
- Information Systems for Biotechnology – http://www.isb.vt.edu (*A National Resource in Agbiotech)*

Additional Websites for Risk Assessment of Organisms

- Belgium - Risk classification of organisms (human, animal and plant pathogens)

(Moniteur Belge 26.02.2002) http://www.biosafety.be/

- International - ABSA compilation of Risk Group classification for infectious substances http://www.absa.org/resriskgroup.html
- UK - Health Directorate, Health and Safety Executive (HSE). The Approved list of Biological Agents. 2004. http://www.hse.gov.uk/pubns/misc208.pdf

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- Department of Health and Human Services. Centers for Disease Control (CDC). 42 CFR 73 Possession, Use, and Transfer of Select Agents and Toxins; Interim Final Rule – http://www.cdc.gov/od/sap/docs/42cfr73.pdf

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Miscellaneous

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APPENDIX 1. DEFINITIONS

From BMBL 4th edition, Appendix F: Definitions (CDC/NIH,1999)

BIOSAFETY: Development and implementation of administrative policies, work practices, facility design, and safety equipment to prevent transmission of biologic agents to workers, other persons, and the environment.

BIOSECURITY: Protection of high-consequence microbial agents and toxins, or critical relevant information, against theft or diversion by those who intend to pursue intentional misuse.

BIOLOGIC TERRORISM: Use of biologic agents or toxins (e.g., pathogenic organisms that affect humans, animals, or plants) for terrorist purposes.

RESPONSIBLE OFFICIAL: A facility official who has been designated the responsibility and authority to ensure that the requirements of Title 42, CFR, Part 73, are met.

RISK: A measure of the potential loss of a specific biologic agent of concern, on the basis of the probability of occurrence of an adversary event, effectiveness of protection, and consequence of loss.

SELECT AGENT: Specifically regulated pathogens and toxins as defined in Title 42, CFR, Part 73, including pathogens and toxins regulated by both DHHS and USDA (i.e., overlapping agents or toxins).

THREAT: The capability of an adversary, coupled with intentions, to undertake malevolent actions.

THREAT ASSESSMENT: A judgment, based on available information, of the actual or potential threat of malevolent action.

VULNERABILITY: An exploitable capability, security weakness, or deficiency at a facility. Exploitable capabilities or weaknesses are those inherent in the design or layout of the biologic laboratory and its protection, or those existing because of the failure to meet or maintain prescribed security standards when evaluated against defined threats.

VULNERABILITY ASSESSMENT: A systematic evaluation process in which qualitative and quantitative techniques are applied to arrive at an effectiveness level for a security system to protect biologic laboratories and operations from specifically defined acts that can oppose or harm a person's interest.

APPENDIX 2. Classification of Etiologic Agents on the Basis of Hazard (CDC,1974)

The basis for the agent classifications:

Class 1. Agents of no or minimal hazard under ordinary conditions of handling.

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 ordinary laboratory techniques.

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.

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 US when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

Containment and training requirements. Recommendations describing the level of competence and physical containment for working with agents of each Class:

Class 1 .Distribution to all users; no special competence or containment required.
(This recommendation still applies for healthy human adults, but host factors must be taken into account when working with opportunistic pathogens, thus good microbiological practices must be learned and used)

Class 2.Distribution to laboratories whose staffs have levels of competency equal to or greater than one would expect in a college department of microbiology. Requests for agents in Class 2 are placed on institutional letterhead. (This requirement assumes that the institution has determined worker competency and facility acceptability; an erroneous assumption).

Class 3. Distribution to laboratories whose staffs have levels of competency equal to or greater than one would expect in a college department of microbiology and who have had special training in handling dangerous agents and are supervised by competent scientists. For aerosol studies, passage in animals, and infection of arthropod vectors, the laboratory should be located in a geographical area in which the chance of accidental establishment of the agent in a susceptible ecologic focus is minimal. Requests for agents in Class 3 are signed by the chairman of the department or the head of the laboratory or research institute where the work will be carried out. Conditions for containment include:

1. A controlled access facility: suite or room separated from the activities of individuals not engaged in handling Class 3 agents and form the general traffic pattern of the rest of the building or laboratory.
2. Negative air pressure is maintained at the site of work n a preparation cubicle or under a hood. Air is recirculated only after it has been adequately decontaminated through high efficiency filters.
3. Animal experiments, including cage sterilization, refuse handling, disposal of animals, etc., are conducted with a level of precaution equivalent to conditions required for laboratory experiments.
4. Personnel at risk are immunized against agents for which immune prophylaxis is available.

Class 4. Distribution to laboratories whose staffs have levels of competency equal to or greater than one would expect in a college department of microbiology and who have had special training in handling dangerous pathogens and are supervised by competent scientists. For aerosol studies, passage in animals, and infection of arthropod vectors, the laboratory should be located in a geographical area in which the chance of accidental establishment of the agent in a susceptible ecologic focus is minimal. Requests for agents in Class 4 are signed by the director of the institute or laboratory where the work is to be carried out. Conditions for containment include all those required for Class 3 agents and the following:

1. Work areas are in a facility which is in effect a separate building, or they are separated from other work areas by effective airlocks.
2. If the work area is not in a separate building, the entire area used for Class 4 agents has a separate air exhaust and negative pressure with respect to other areas of the building. Exhaust air is decontaminated by filtration through high efficiency filters or by some other suitable process. Class 4 agents are manipulated only in safety cabinets equipped with absolute filters.
3. Access to work areas is restricted to individuals immunized or otherwise under specific control.
4. Protective clothing is worn, and it is decontaminated before being removed from the laboratory area.
5. When an agent is used in entomological experiments, the windows, walls, floor, ceiling, and airlock of the work area are insect-proof, and pure pyrethrum insecticide or a suitable insect killing device is available in the airlock.

APPENDIX 3. Risk Classification Criteria for World Health Organization (WHO), Australia, Canada, European Union (EU), and for the USA, the NIH for RDNA and the CDC/NIH.

1. **WHO Classification of Infective Microorganisms by Risk Group (2004)**. WHO Basis for Risk Grouping: Each country classifies the agents in that country by risk group based on pathogenicity of the organism, modes of transmission and host range of the organism. These may be influenced by existing levels of immunity, density and movement of host population presence of appropriate vectors and standards of environmental hygiene.

    - Availability of effective preventive measures. Such measures may include: prophylaxis by vaccination or antisera; sanitary measures, e.g. food and water hygiene; the control of animal reservoirs or arthropod vectors; the movement of people or animals; and the importation of infected animals or animal products.
    - Availability of effective treatment. This includes passive immunization and post-exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of the emergence of resistant strains. It is important to take prevailing conditions in the geographical area in which the microorganisms are handled into account. Note: Individual governments may decide to prohibit the handling or importation of certain pathogens except for diagnostic purposes.

    - **WHO Risk Group 1** (no or low individual and community risk). A microorganism that is unlikely to cause human disease or animal disease
    - **WHO Risk Group 2** (moderate individual risk, low community risk). A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.
    - **WHO Risk Group 3** (high individual risk, low community risk). A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.
    - **WHO Risk Group 4** (high individual and community risk). A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

2. **Australian/New Zealand Standard (2002).** Standard AS/NZS 2243.3:2002. Safety in laboratories Part 3: Microbiological aspects and containment facilities.. The following classification is based on the pathogenicity of the agent, the mode of transmission and host range of the agent, the availability of effective preventive measures and the availability of effective treatment.

- **Group 1** (low individual and community risk). A microorganism that is unlikely to cause human, plant or animal disease.
- **Group 2** (moderate individual risk, limited community risk). A pathogen that can cause human, animal or plant disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause infection, but effective treatment and preventive measures are available and the risk of spread is limited.
- **Group 3** (high individual risk, limited community risk). A pathogen that usually causes serious human or animal disease and may present a serious hazard to laboratory workers. It could present a risk if spread in the community or the environment, but there are usually effective preventive measures or treatment available.
- **Group 4** (high individual and community risk). A pathogen that usually produces life-threatening human or animal disease represents a serious hazard to laboratory workers and is readily transmissible from one individual to another. Effective treatment and preventive measures are not usually available.

3. **Canadian Laboratory Safety Guidelines (2004; list not available)** Inherent risks of a pathogen made on basis of factors such as severity of disease caused, routes of infection, virulence and infectivity takes into account existence of effective therapies, possibilities for immunization, presence of vectors, quantity of agent and whether agent is indigenous to Canada, possible effects on other species, including plants, or possible economic environmental effects.
   - **Risk Group 1** (low individual and community risk). Any biological agent that is unlikely to cause disease in healthy workers or animals.
   - **Risk Group 2** (moderate individual risk, limited community risk). Any pathogen that can cause human disease, but under normal circumstances is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease, effective treatment and preventive measures are available and the risk of spread is limited.
   - **Risk Group 3** (high individual risk, low community risk). Any pathogen that usually causes serious human disease, or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes disease treatable by antimicrobial or antiparasitic agents.
   - **Risk Group 4** (high individual and community risk). Any pathogen that usually produces very serious human disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa, directly or indirectly, or by casual contact.

4. **European Economic Community (2000)**. Directive 2000/54/EC and Directive 90/679/EEC (adopted 20 November, 1990; revised 18 September 2000) on the protection of workers from risks related to exposure to biological agents at work provides for the Classification of biological agents into four infection risk groups on the basis of the following criteria:

   - **Group 1** biological agent means one that is unlikely to cause human disease.
   - **Group 2** biological agent means one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community; there is usually effective prophylaxis or treatment available.
   - **Group 3** biological agent means one that can cause severe human disease and present a serious hazard to workers; it may present a risk of spreading to the community, but there is usually effective prophylaxis or treatment available.
   - **Group 4** biological agent means one that causes severe human disease and is a serious hazard to workers; it may present a high risk of spreading to the community; there is usually no effective prophylaxis or treatment available. (See also Official Journal of the European Communities No L262/21 dated September 18, 2000.) Article 2. Definitions; Article 18. Classification of biological agents; Annex III. Community Classification. Introductory Notes)

5. **NIH Recombinant DNA Guidelines** (**USA, 2002).** April 2002. Appendix B. http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html

   - **Risk Group 1** (RG1) Agents that are not associated with disease in healthy adult humans. Includes a list of animal viral etiologic agents in common use.
   - **Risk Group 2** (RG2) Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are often available.
   - **Risk Group 3** (RG3) Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
   - **Risk Group 4** (RG4) Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

6. **CDC/NIH Guidelines (1999).** "Biosafety in Microbiological and Biomedical Laboratories" 4th Edition, 1999. Section III gives criteria for placing work into a biosafety containment level; not yet translated into Risk groups.

   - **Biosafety Level 1 (BSL 1)**: well characterized agents not consistently known to cause disease in healthy adult humans, of minimal potential hazard to laboratory personnel and the environment
   - **Biosafety Level 2 (BSL 2)**: agents of moderate potential hazard to personnel and the environment

- **Biosafety Level 3 (BSL 3)**: indigenous or exotic agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route (applicable to clinical, diagnostic, teaching, research or production facilities)
- **Biosafety Level 4 (BSL 4)**: dangerous and exotic agents which pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease

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APPENDIX 4. Biosafety in Biomedical and Microbiological Laboratories

BMBL Section V Risk Assessment

"Risk" implies the probability that harm, injury, or disease will occur. In the context of the microbiological and biomedical laboratories, the assessment of risk focuses primarily on the prevention of laboratory-associated infections. When addressing laboratory activities involving infectious or potentially infectious material, risk assessment is a critical and productive exercise. It helps to assign the biosafety levels (facilities, equipment, and practices) that reduce the worker's and the environment's risk of exposure to an agent to an absolute minimum. The intent of this section is to provide guidance and to establish a framework for selecting the appropriate biosafety level.

Risk assessment can be qualitative or quantitative. In the presence of known hazards (e.g., residual levels of formaldehyde gas after a laboratory decontamination), quantitative assessments can be done. But in many cases, quantitative data will be incomplete or even absent (e.g., investigation of an unknown agent or receipt of an unlabeled sample). Types, subtypes, and variants of infectious agents involving different or unusual vectors, the difficulty of assays to measure an agent's amplification potential, and the unique considerations of genetic recombinants are but a few of the challenges to the safe conduct of laboratory work. In the face of such complexity, meaningful quantitative sampling methods are frequently unavailable. Therefore, the process of doing a risk assessment for work with biohazardous materials cannot depend on a prescribed algorithm.

The laboratory director or principal investigator is responsible for assessing risks in order to set the biosafety level for the work. This should be done in close collaboration with the Institutional Biosafety Committee (and/or other biosafety professionals as needed) to ensure compliance with established guidelines and regulations.

In performing a qualitative risk assessment, all the risk factors are first identified and explored. There may be related information available, such as this manual, the *NIH Recombinant DNA Guidelines*, the *Canadian Laboratory Biosafety Guidelines*, or the *WHO Biosafety Guidelines*. In some cases, one must rely on other sources of information such as field data from subject matter experts. This information is interpreted for its tendency to raise or lower the risk of laboratory-acquired infection.(1)

The challenge of risk assessment lies in those cases where complete information on these factors is unavailable. A conservative approach is generally advisable when insufficient information forces subjective judgment. Universal precautions are always advisable.

The factors of interest in a risk assessment include:

- The *pathogenicity* of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease). The more severe the potentially acquired disease, the

higher the risk. For example, *staphylococcus aureus* only rarely causes a severe or life threatening disease in a laboratory situation and is relegated to BSL-2. Viruses such as Ebola, Marburg, and Lassa fever, which cause diseases with high mortality rates and for which there are no vaccines or treatment, are worked with at BSL-4. However, disease severity needs to be tempered by other factors. Work with human immunodeficiency virus (HIV) and hepatitis B virus is also done at BSL-2, although they can cause potentially lethal disease. But they are not transmitted by the aerosol route, the incidence of laboratory-acquired infection is extremely low for HIV, and an effective vaccine is available for hepatitis B.

- The *route of transmission* (e.g., parenteral, airborne, or by ingestion) of newly isolated agents may not be definitively established. Agents that can be transmitted by the aerosol route have caused most laboratory infections. It is wise, when planning work with a relatively uncharacterized agent with an uncertain mode of transmission, to consider the *potential for aerosol transmission.* The greater the aerosol potential, the higher the risk.
- *Agent stability* is a consideration that involves not only aerosol infectivity (e.g., from spore-forming bacteria), but also the agent's ability to survive over time in the environment. Factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants must be considered.
- The *infectious dose* of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker, and may pose a serious risk to those with lesser resistance. The laboratory worker's *immune* status is directly related to his/her susceptibility to disease when working with an infectious agent.
- The *concentration* (number of infectious organisms per unit volume) will be important in determining the risk. Such a determination will include consideration of the milieu containing the organism (e.g., solid tissue, viscous blood or sputum, or liquid medium) and the laboratory activity planned (e.g., agent amplification, sonication, or centrifugation). The volume of concentrated material being handled is also important. In most instances, the risk factors increase as the working volume of high-titered microorganisms increases, since additional handling of the materials is often required.
- The *origin* of the potentially infectious material is also critical in doing a risk assessment. "Origin" may refer to geographic location (e.g., domestic or foreign); host (e.g., infected or uninfected human or animal); or nature of source (potential zoonotic or associated with a disease outbreak). From another perspective, this factor can also consider the potential of agents to endanger American livestock and poultry.
- The *availability of data from animal studies*, in the absence of human data, may provide useful information in a risk assessment. Information about pathogenicity, infectivity, and route of transmission in animals may provide valuable clues. Caution must always be exercised, however, in translating infectivity data from one species of animal to another species.
- The established *availability of an effective prophylaxis* or therapeutic intervention is another essential factor to be considered. The most common form of

prophylaxis is immunization with an effective vaccine. Risk assessment includes determining the availability of effective immunizations. In some instances, immunization may affect the biosafety level (e.g., the BSL-4 Junin virus can be worked on at BSL-3 by an immunized worker). Immunization may also be passive (e.g., the use of serum immunoglobulin in HBV exposures). However important, immunization only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Occasionally, immunization or therapeutic intervention (antibiotic or antiviral therapy) may be particularly important in field conditions. The offer of immunizations is part of risk management.

- *Medical surveillance* ensures that the safeguards decided upon in fact produce the expected health outcomes. Medical surveillance is part of risk management. It may include serum banking, monitoring employee health status, and participating in post-exposure management.
- Risk assessment must also include an evaluation of the *experience and skill level of at-risk personnel* such as laboratorians and maintenance, housekeeping, and animal care personnel (see Section III). Additional education may be necessary to ensure the safety of persons working at each biosafety level.

The infectious agents whose risk is evaluated often will fall into the following discrete categories:

- **Materials containing known infectious agents**. The characteristics of most known infectious agents have been well identified. Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies. Infectious agents known to have caused laboratory-associated infections are included in this volume's agent summary statements (see Section VII). Other sources include the American Public Health Association's manual, *Control of Communicable Diseases*.(2) Literature reviews on laboratory acquired infections also may be helpful.(3)(4)(5)(6)(7)(8)

- **Materials containing unknown infectious agents.** The challenge here is to establish the most appropriate biosafety level with the limited information available. Often these are clinical specimens. Some questions that may help in this risk assessment include:

  1. Why is an infectious agent suspected?
  2. What epidemiological data are available? What route of transmission is indicated? What is the morbidity or mortality rate associated with the agent?
  3. What medical data are available?

  The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In the absence of hard data, a conservative approach is advisable.

- **Materials containing recombinant DNA molecules.** This category of agents includes microorganisms that have been genetically modified through recombinant DNA technologies. These technologies continue to evolve rapidly. Experimental procedures designed to derive novel recombinant viruses, bacteria, yeast, and other microorganisms have become commonplace in recent years. It is highly likely that future applications of recombinant DNA technology will produce new hybrid viruses. The National Institutes of Health publication, *Guidelines for Research Involving Recombinant DNA Molecules*,(9) is a key reference in establishing an appropriate biosafety level for work involving recombinant microorganisms.

In selecting an appropriate biosafety level for such work, perhaps the greatest challenge is to evaluate the potential increased biohazard associated with a particular genetic modification. In most such cases, the selection of an appropriate biosafety level begins by establishing the classification of the non-modified virus. Among the recombinant viruses now routinely developed are adenoviruses, alphaviruses, retroviruses, vaccinia viruses, herpesviruses, and others designed to express heterologous gene products. However, the nature of the genetic modification and the quantity of virus must be carefully considered when selecting the appropriate biosafety level for work with a recombinant virus.

Among the points to consider in work with recombinant microorganisms are:

1. Does the inserted gene encode a known toxin or a relatively uncharacterized toxin?
2. Does the modification have the potential to alter the host range or cell tropism of the virus?
3. Does the modification have the potential to increase the replication capacity of the virus?
4. Does the inserted gene encode a known oncogene?
5. Does the inserted gene have the potential for altering the cell cycle?
6. Does the viral DNA integrate into the host genome?
7. What is the probability of generating replication-competent viruses?

This list of questions is not meant to be inclusive. Rather, it serves as an example of the information needed to judge whether a higher biosafety level is needed in work with genetically modified microorganisms. Since in many cases the answers to the above questions will not be definitive, it is important that the organization have a properly constituted and informed Institutional Biosafety Committee, as outlined in the NIH guidelines, to evaluate the risk assessment.

- **Materials that may or may not contain unknown infectious agents.** In the absence of information that suggests an infectious agent, universal precautions are indicated.

- **Animal studies.** Laboratory studies involving animals may present many different kinds of physical, environmental, and biological hazards. The specific

hazards present in any particular animal facility are unique, varying according to the species involved and the nature of the research activity. The risk assessment for biological hazard should particularly focus on the animal facility's potential for increased exposure, both to human pathogens and to zoonotic agents.

The animals themselves can introduce new biological hazards to the facility. Latent infections are most common in field-captured animals or in animals coming from unscreened herds. For example, monkey b-virus presents a latent risk to individuals who handle macaques. The animal routes of transmission must also be considered in the risk assessment. Animals that shed virus through respiratory dissemination or dissemination in urine or feces are far more hazardous than those that do not. Animal handlers in research facilities working on infectious agents have a greater risk of exposure from the animals' aerosols, bites, and scratches. Section IV describes the practices and facilities applicable to work on animals infected with agents assigned to corresponding Biosafety Levels 1-4.(1)

- **Other applications** The described risk assessment process is also applicable to laboratory operations other than those involving the use of primary agents of human disease. It is true that microbiological studies of animal host-specific pathogens, soil, water, food, feeds, and other natural or manufactured materials, pose comparatively lower risks for the laboratory worker. Nonetheless, microbiologists and other scientists working with such materials may find the practices, containment equipment, and facility recommendations described in this publication of value in developing operational standards to meet their own assessed needs.

- **Other Resources** *NIH Guidelines for Recombinant DNA Molecules*: http://www.nih.gov/od/orda/toc.htm NIH Office of Recombinant DNA Activities: http://www.nih.gov/od/orda

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APPENDIX 5. NIH Guidelines for Research Involving Recombinant DNA Molecules (April 2002) Section II Risk Assessment; Appendix G (Lab scale) and K (Large Scale) NIH, 2002

Section II-B. 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-B, Footnotes and References of Sections I-IV). 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, Physical Containment. 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, Biological Containment).

NIH APPENDIX G: Physical Containment

Appendix G specifies physical containment for standard laboratory experiments and defines Biosafety Level 1through Biosafety Level 4. For large-scale (over 10 liters) research or production, Appendix K (*Physical Containment for Large Scale Uses of*

Organisms Containing Recombinant DNA Molecules) supersedes Appendix G. Appendix K defines Good Large Scale Practice through Biosafety Level 3 - Large Scale. For certain work with plants, Appendix P (*Physical and Biological Containment for Recombinant DNA Research Involving Plants*) supersedes Appendix G. Appendix P defines Biosafety Levels 1 through 4 - Plants. For certain work with animals, Appendix Q (*Physical and Biological Containment for Recombinant DNA Research Involving Animals*) 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, *Footnotes and References of Appendix G*). Consequently, all personnel directly or indirectly involved in experiments using recombinant DNA shall receive adequate instruction (see Sections IV-B-1-h, *Responsibilities of the Institution--General Information*, and IV-B-7-d, *Responsibilities of the Principal Investigator Prior to Initiating Research*). 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-7-d, *Responsibilities of the Principal Investigator Prior to Initiating Research* and IV-B-7-e, *Responsibilities of the Principal Investigator During the Conduct of the Research*). 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, *Responsibilities of the Institution--General Information*).

The *Laboratory Safety Monograph* (see Appendix G-III-O, *Footnotes and References of Appendix G*) and *Biosafety in Microbiological and Biomedical Laboratories* (see Appendix G-III-B, *Footnotes and References of Appendix G*) 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, *Footnotes and References of Appendix G*).

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, *Footnotes and References of Appendix G*). 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.

NIH 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-D-6, *Experiments Involving More than 10 Liters of Culture*. 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, *Physical Containment*, 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, *Biological Safety Officer*; (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, *Experiments Covered by the NIH Guidelines.* 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, *Exemptions under Section III-F-6,* 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.

Page 13 - NIH Guidelines for Research Involving Recombinant DNA Molecules (April 2002) 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, Physical Containment, 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, Biological Containment, 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, Physical and Biological Containment for Recombinant DNA Research Involving Plants. 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, Physical and Biological Containment for Recombinant DNA Research Involving Animals. 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.

Page 14 - NIH Guidelines for Research Involving Recombinant DNA Molecules (April 2002). 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.

APPENDIX K. *Physical Containment for Large Scale Uses of Organisms Containing Recombinant DNA Molecules 1*

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, and 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.

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 Laboratory Director. 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, *Physical Containment Levels--Biosafety Level 1*, 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. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

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 except when the culture fluids contain viable organisms or vectors intended as final product as described in Appendix K-III-C above. 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), *Institutional Biosafety Committee*, and IV-B-3-c-(3), *Biological Safety Officer*, 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/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). 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, *Physical Containment Levels--Biosafety Level 2*, 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. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

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 except when the culture fluids contain viable organisms or vectors intended as final product as described in Appendix K-IV-C above. 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), *Institutional Biosafety Committee*, and IV-B-3-c-(3), *Biological Safety Officer*, 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/OBA, and other appropriate authorities (if applicable). Reports to NIH/OBA shall be sent to the Office of Biotechnology Activities, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985 (20817 for non-USPS mail), 301-496-9838, 301-496-9839 (fax). 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, *Physical Containment Levels--Biosafety Level 3*, 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. Culture fluids that contain viable organisms or viral vectors intended as final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.

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 except when the culture fluids contain viable organisms or vectors intended as final product as described in Appendix K-V-C above. 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), *Institutional Biosafety Committee,* and IV-B-3-c-(3), *Biological Safety Officer*, 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-O-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-VI. Footnotes of Appendix K

Appendix K-VII. Definitions

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 6. Agents requiring BSL 3 Ag for work with loose animals

- Avian influenza virus (highly pathogenic)
- African swine fever virus
- Classical swine fever
- Foot and mouth disease virus
- Lumpy skin disease virus
- Mycoplasma mycoides subsp. mycoides, (small colony type)
- Mycoplasma capricolum
- Newcastle disease virus (velogenic strains)
- Peste des petits ruminants (plague of small ruminants)

- Rift Valley fever virus
- Rinderpest virus

APPENDIX 7. Laboratory acquired infections between 1979-2004 (From Harding and Byers, 2006.in press)

Category of Agent	**Symptomatic LAIs[a]**	**Asymptomatic LAIs[a]**	**Total primary LAIs**	**# Deaths**	**2nd Infections**	**# Publications**
Bacteria	598	60	658	17[b]	7	125
Rickettsia	187	214	401	1	0	13
Viruses	608	430	1,038	18[c]	10	97
Parasites	49	4	53	0	0	30
Fungi	6	0	6	0	0	5
Total	1,448	708	2,156	36	17	270

[a]LAIs resulting from primary infections; secondary infections not included in totals.

[b]Four deaths were attributed to aborted fetuses resulting from *Brucella melitensis* exposures and 1 to a secondary contact exposed to a multi-drug resistant *Salmonella agoni*.

[c]One death was attributed to an aborted fetus associated with Parvovirus infection and 1 to a secondary contact exposed to SARS.

APPENDIX 8. Risk assessment matrix for agent hazards*

RISK FACTORS / AGENT HAZARDS	DEGREE OF LABORATORY RISK		
	LOW TO MODERATE	**MODERATE TO HIGH**	**HIGH**
Pathogenicity	Mild to moderate disease (*Salmonella typhimurium*)	Moderate to serious disease (*Mycobacterium tuberculosis*)	Severe disease (Cercopithecine herpes virus)
Virulence	Mild to moderate disease or low infectivity	Severe disease or moderate infectivity	Lethal disease or high infectivity
Infective dose	$>10^6$ IU (*Vibrio cholerae*)	10^6 – 100 IU (Influenza A virus)	<100 IU (*Francisella tularensis*}
Transmission	Indirect contact (contact with contaminated surfaces, animal bedding)	Direct contact (droplet, tissue, fluid, secretion contact with mucous membranes; ingestion)	Inhalation or percutaneous inoculation (needle stick)
Stability	Survive minutes to hours on surfaces (Measles virus)	Survive days to weeks on surfaces (Hepatitis B virus)	Survive weeks to months in hostile environment (*Coxiella burnetii*)
Animal host range	Not likely to cross species barrier	Broad host range but not known to cause disease in humans	Zoonoses (Hanta virus)
Occurrence of natural disease	Endemic	Not endemic	Importation controlled by CDC or USDA
Probable causes of laboratory-associated infections	Absence of LAI reports	Accidents; percutaneous; ingestion; unknown	Evidence of inhalation transmission
WHO Risk Group**	Risk Group 2 (moderate individual risk, low community risk)	Risk Group 3 (high individual risk, low community risk)	Risk Group 4 (high individual and community risk)

*adapted from W. E. Barkley, personal communication

** See WHO RG definitions in Appendix 3.

APPENDIX 9. RISK ASSESSMENT MATRIX FOR PROTOCOL HAZARDS*

Protocol Hazards	Low Risk	Moderate Risk	High Risk
Agent Concentration[a]	$<10^3$ IU/ml	$10^3 - 10^6$ IU/ml	$>10^9$ IU/ml
Suspension volume	<1 ml	1 ml – 1 L	>1L
Equipment/procedures that generate droplets and 2-10 μm particle aerosols	Streaking "smooth" agar on a Petri dish	Opening blender lid after 1 min; pipetting with minimal bubbles; Streaking "rough" agar on a Petri dish	Opening blender lid after stop; Flaming an inoculating loop; pipetting with bubbles
Protocol Complexity	Standard repetitive procedures	Periodic change in procedures	Frequent change and complex procedures
Use of Animals	Use of safe animal care practices	Necropsies; large animals handling	Aerosol challenge protocols
Use of Sharps		With protective devices; safety sharps	Without protective devices

*adapted from W. E. Barkley, personal communication

[a]The risk related to the agent concentration depends upon the infectious dose, which can be very small. (for Coxiella burnetti high risk is 1-10 IU).

APPENDIX 10. Concentration and particle size of aerosols created during representative laboratory techniques[a]

Operation	No. of viable colonies[b]	Particle size[c] (μm)
Mixing culture with:		
Pipet	6.6	2.3 ± 1.0
Vortex mixer (15 sec.)	0.0	0.0
Mixer overflow	9.4	4.8 ± 1.9
Use of blender:		
Top on	119.6	1.9 ± 0.7
Top off	1,500.0	1.7 ± 0.5
Use of a sonicator	6.3	4.8 ± 1.6
Lyophilized cultures:		
Opened carefully	134.0	10.0 ± 4.3
Dropped and broken	4,838.0	10.0 ± 4.8

[a]Adapted from Kenny and Sabel,1968.

[b]Mean number of viable colonies per cubic foot of air sampled.

[c]Count median diameter of particle.

(Table 3 from Harding and Byers, 2006)

APPENDIX 11. Risk assessment matrix for Susceptibility to Disease*

RISK FACTORS	DEGREE OF LABORATORY RISK		
	LOW TO MODERATE	MODERATE TO HIGH	HIGH
Susceptibility to disease			
Potential for exposure	Non-lab person associated with the lab; Intermittent visitor to lab	Lab worker in room where agent is handled	Lab worker who handles agent
Individual susceptibility	Effective immunization	Competent immune status	Compromised immune status
Availability of effective vaccine or other protective prophylaxis	Yes	Less effective prophylaxis	No
Availability of effective treatments and therapeutic agents	Yes	Treatments and therapeutic agents offer some value	No

*adapted from W. E. Barkley, personal communication