

APPENDIX K. PHYSICAL CONTAINMENT FOR LARGE SCALE USES OF ORGANISMS CONTAINING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES

Appendix K specifies physical containment guidelines for large-scale (greater than 10 liters of culture) research or production involving viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid molecules. 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules which require Biosafety Level (BL) 3 containment at the laboratory scale. The program shall include: pre assignment 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 or synthetic nucleic acid molecule 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 or synthetic nucleic acid molecule 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 or synthetic 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-8*](#), 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules that require BL3 containment at the laboratory scale. No provisions are made for large-scale research or production of viable organisms containing recombinant or synthetic nucleic acid molecules that require BL4 containment at the laboratory scale. If necessary, these requirements will be established by NIH on an individual basis.

Appendix K-II. Good Large Scale Practice (GLSP)

Appendix K-II-A. Institutional codes of practice shall be formulated and implemented to assure adequate control of health and safety matters.

Appendix K-II-B. Written instructions and training of personnel shall be provided to assure that cultures of viable organisms containing recombinant or synthetic nucleic acid molecules are handled prudently and that the work place is kept clean and orderly.

Appendix K-II-C. In the interest of good personal hygiene, facilities (e.g., hand washing sink, shower, changing room) and protective clothing (e.g., uniforms, laboratory coats) shall be provided that are appropriate for the risk of exposure to viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules to the environment.

Appendix K-III-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH OSP, and other appropriate authorities (if applicable). Reports to NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available [here](#) and on the [OSP website](#) (www.osp.od.nih.gov). Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Appendix K-IV-B. Cultures of viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules to the environment.

Appendix K-IV-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules shall be tested for integrity of the containment features using the organism that will serve as the host for propagating recombinant or synthetic nucleic acid molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules are immediately reported to the Biological Safety Officer, Institutional Biosafety Committee, NIH OSP, and other appropriate authorities (if applicable). Reports to NIH OSP shall be sent to the Office of Science Policy, National Institutes of Health, preferably by e-mail to: NIHGuidelines@od.nih.gov; additional contact information is also available [here](#) and on the [OSP website](#) (www.osp.od.nih.gov). Medical evaluation, surveillance, and treatment are provided as

appropriate and written records are maintained.

Appendix K-V-B. Cultures of viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules to the environment.

Appendix K-V-F. A closed system or other primary containment equipment that has contained viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid molecules.

Appendix K-V-G. A closed system used for the propagation and growth of viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules and other primary containment equipment used to contain operations involving viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid molecules shall be tested for integrity of the containment features using the organisms that will serve as the host for propagating the recombinant or synthetic nucleic acid molecules. Testing shall be accomplished prior to the introduction of viable organisms containing recombinant or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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 or synthetic nucleic acid 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. Service 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 or synthetic nucleic acid 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 or synthetic nucleic acid molecules.

Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL) - Large Scale (LS) Practice (See [Appendix K-VI-A](#), *Footnotes Of Appendix K*)

CRITERION [See Appendix K-VI-B , <i>Footnotes of Appendix K</i>]		GLSP	BL1-LS	BL2-LS	BL3-LS
1.	Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures.	K-II-A	G-I		
2.	Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees.	K-II-B	G-I		
3.	Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work.	K-II-C	G-II-A-1-h	G-II-B-2-f	G-II-C-2-i
4.	Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place.	K-II-C	G-II-A-1-d G-II-A-1-e	G-II-B-1-d G-II-B-1-e	G-II-C-1-c G-II-C-1-d
5.	Internal accident reporting.	K-II-G	K-III-A	K-IV-A	K-V-A
6.	Medical surveillance.	NR	NR		
7.	Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment).	NR	K-III-B	K-IV-B	K-V-B
8.	Culture fluids not removed from a system until organisms are inactivated.	NR	K-III-C	K-IV-C	K-V-C
9.	Inactivation of waste solutions and materials with respect to their biohazard potential.	K-II-E	K-III-C	K-IV-C	K-V-C
10.	Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system.	Minimize <i>Procedure</i> K-II-F	Minimize <i>Engineer</i> K-III-B K-III-D	Prevent <i>Engineer</i> K-IV-B K-IV-D	Prevent <i>Engineer</i> K-V-B K-V-D
11.	Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms.	NR	Minimize K-III-E	Prevent K-IV-E	Prevent K-V-E
12.	Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure.	NR	K-III-F	K-IV-F	K-V-F
13.	Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features.	NR	NR	NR	K-V-G
14.	Rotating seals and other penetrations into closed system designed to prevent or minimize leakage.	NR	NR	Prevent K-IV-G	Prevent K-V-H
15.	Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment.	NR	NR	K-IV-H	K-V-I
16.	Validated integrity testing of closed containment system.	NR	NR	K-IV-I	K-V-J
17.	Closed system to be permanently identified for record keeping purposes.	NR	NR	K-IV-J	K-V-K

CRITERION [See Appendix K-VI-B, Footnotes of Appendix K]		GLSP	BL1-LS	BL2-LS	BL3-LS
18.	Universal biosafety sign to be posted on each closed system.	NR	NR	K-IV-K	K-V-L
19.	Emergency plans required for handling large losses of cultures.	K-II-G	K-III-G	K-IV-L	K-V-M
20.	Access to the work place.	NR	G-II-A-1-a	G-II-B-1-a	K-V-N
21.	Requirements for controlled access area.	NR	NR	NR	K-V-N&O

NR = not required

Appendix K-VI. Footnotes of Appendix K

Appendix K-VI-A. This table is derived from the text in Appendices G (*Physical Containment*) and K and is not to be used in lieu of Appendices G and K.

Appendix K-VI-B. The criteria in this grid address only the biological hazards associated with organisms containing recombinant or synthetic nucleic acid. Other hazards accompanying the large-scale cultivation of such organisms (e.g., toxic properties of products; physical, mechanical, and chemical aspects of downstream processing) are not addressed and shall be considered separately, albeit in conjunction with this grid.

Appendix K-VII. Definitions to Accompany Containment Grid and Appendix K

Appendix K-VII-A. Accidental Release. An accidental release is the unintentional discharge of a microbiological agent (i.e., microorganism or virus) or eukaryotic cell due to a failure in the containment system.

Appendix K-VII-B. Biological Barrier. A biological barrier is an impediment (naturally occurring or introduced) to the infectivity and/or survival of a microbiological agent or eukaryotic cell once it has been released into the environment.

Appendix K-VII-C. Closed System. A closed system is one in which by its design and proper operation, prevents release of a microbiological agent or eukaryotic cell contained therein.

Appendix K-VII-D. Containment. Containment is the confinement of a microbiological agent or eukaryotic cell that is being cultured, stored, manipulated, transported, or destroyed in order to prevent or limit its contact with people and/or the environment. Methods used to achieve this include: physical and biological barriers and inactivation using physical or chemical means.

Appendix K-VII-E. De minimis Release. *De minimis* release is the release of: (i) viable microbiological agents or eukaryotic cells that does not result in the establishment of disease in healthy people, plants, or animals; or (ii) in uncontrolled proliferation of any microbiological agents or eukaryotic cells.

Appendix K-VII-F. Disinfection. Disinfection is a process by which viable microbiological agents or eukaryotic cells are reduced to a level unlikely to produce disease in healthy people, plants, or animals.

Appendix K-VII-G. Good Large Scale Practice Organism. For an organism to qualify for Good Large Scale Practice consideration, it must meet the following criteria [Reference: Organization for Economic Cooperation and Development, *Recombinant DNA Safety Considerations*, 1987, p. 34-35]: (i) the host organism should be non-pathogenic, should not contain adventitious agents and should have an extended history of safe large-scale use or have built-in environmental limitations that permit optimum growth in the large-scale setting but limited survival without adverse consequences in the environment;

(ii) the recombinant or synthetic nucleic acid molecule-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.
