

THE GUIDELINES EXPLAINED (April 2002 Guidelines Version)¹

The next few pages were written to extract the essence of the NIH guideline requirements and put them into readable form. Since many details are omitted (and the devil is in the details) the actual guidelines should be consulted when in doubt. Plant, whole animal, and human experiments are given special treatment in the guidelines (and in this explanation).

The NIH recombinant DNA guidelines are an intimidating publication. They are long, full of legalese, and have lots of cross references, exceptions, lists, sections, appendices, and tables. No one actually reads the guidelines cover to cover. But they are legally binding on any institution using NIH funds.

PREREQUISITES

To make sense of the following, the reader should know the basic ideas behind Biosafety Levels 1 through 4. For the NIH Biosafety Levels are based on four risk groups. Risk groups are summarized on page 2. A summary table giving the characteristics of the four laboratory Biosafety Levels is on page 7. Animal Biosafety Levels are summarized on page 8.

REGISTRATION

The NIH requires all labs working with recombinant DNA register with their local Institutional Biosafety Committee. The guidelines distinguish among five kinds of registrations. The type depends on the potential hazard of the work. More hazardous means more approvals are needed. The five types are:

- Work that cannot begin until there is NIH and IBC approval (Sections III-A and III-B), this is the most dangerous level and it is [TABOO] (page 3)
- Work that cannot begin until there is IBC approval and RAC review (Section III-C), this tends to be sensitive and potentially dangerous.[WAIT AND WAIT] (page 3). *Human Gene Transfer Studies* are in this category (page 5).
- Work that cannot begin until there is IBC approval (III-D), there is usually a short [WAIT] (page 3). *Human Xenotransplantation Studies* are in this category (this requirement comes from the FDA).
- Work can begin when IBC is notified (Section III-E) [NO WAITING] (page 3)
- Work that is Exempt from NIH guidelines (Section III-F). No approval is needed but the work should be registered with IBC. [NO WAITING PLUS] (page 4)

WHAT IS RECOMBINANT DNA?

The NIH Guidelines define recombinant DNA thus:

"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."

WHAT ARE THE RAC AND THE OBA?

The NIH OBA (Office of Biotechnology Activities) is an administrative arm responsible for carrying out the orders of the NIH Director with regard to recombinant DNA, genetic testing and xenotransplantation. An advisory committee is involved in establishing policies for each of these fields. For recombinant DNA the committee is called the Recombinant DNA Advisory Committee or "RAC".

¹ This version of The Guidelines Explained was revised in June 2009.

RISK GROUPS

The NIH classifies biological agents into four Risk Groups according to their human pathogenicity (see NIH Guidelines, Section II-A-1):

- Risk Group 1 - not associated with disease in healthy adults.
- Risk Group 2 - associated with disease that is rarely serious and for which therapeutic or preventive options are *often* available.
- Risk Group 3 - associated with serious or lethal disease for which therapeutic or preventive options *may* be available.
- Risk Group 4 - associated with serious or lethal disease for which therapeutic or preventive options *not usually* available.

Appendix B in the NIH Guidelines lists a number of biologic agents according to their Risk Group.

In general, the Risk Group determines the Biosafety Level needed: for instance a Risk Group 3 agent is usually studied in a BL3 or BL3-N (animal) lab.

The table on page 6 summarizes the NIH Biosafety Level recommendations according to risk group.

The CDC recommendations for laboratory and animal facilities by biosafety level are summarized on page 7 and 8 respectively.

Things are a-changin'

The concept of Risk Groups as a basis for determining Biosafety Level is rapidly falling out of favor. For one thing, there is no uniform definition of Risk Groups. For another, the system is inflexible. For another, the Risk Group listing in the NIH Guidelines is more than 10 years out of date. Finally, the system has been abandoned in many countries and parts of the US regulatory community. For instance, the CDC, the WHO and Health Canada have abandoned the Risk Group system in their publications.

Nomenclature is also a problem. The NIH uses BL1-BL4 and BL1-N – BL4, the CDC uses BSL1-BSL4 and ABSL1 – ABSL4, and Health Canada uses CL1 – CL4.

“TABOO”
(NIH APPROVAL AND IBC PERMISSION REQUIRED)
(from NIH Guidelines Sections III-A and III-B)

- Making drug resistant constructs of microorganisms if they compromise the drug’s therapeutic potential,
- Making constructs that synthesize vertebrate toxins with an LD₅₀ of 100 ng/Kg or less,
- Making constructs in *E. Coli* that synthesize vertebrate toxins that are “lethal” between 100 ng/Kg and 100:g/Kg

“WAIT AND WAIT”
(NIH REVIEW AND IBC PERMISSION REQUIRED)
(from Section III-C)

- Studies in which genes are transferred into humans must be submitted to the NIH OBA for review. If the OBA find the study to be "novel" it will place the study on the next RAC meeting agenda. IBC approval must wait for the RAC's review. If, on the other hand, OBA does not deem the study to be "novel," IBC can act immediately.

“WAIT”
(IBC APPROVAL NEEDED BEFORE STARTING)
(From Section III-D)

A substantial number of studies carried out at Harvard laboratories are in this group. They are examined by IBC with an eye to recommending safe procedures and containment. To reach a conclusion it is often useful to classify the risk associated with a proposed study according to the risk associated with the organisms to be used. A convenient classification tool is the concept of “risk group.”

“NO WAITING”
JUST NOTIFY IBC AT THE SAME TIME WORK STARTS
(From Section III-E)

COMS DOES NOT RECOGNIZE THIS CATEGORY.

All recombinant DNA studies must be registered with and approved by COMS before they start.

- **“LOW HAZARD” RECOMBINANT DNA** (Section III-E)
 - Non-pathogenic prokaryotes or non-pathogenic lower eukaryotes (use BL1),
 - Recombinant DNA with less than 10% of a eukaryotic viral genome (and no helper) used exclusively in tissue culture (BL1 recommended), containment is recommended when the host is:
 - a non-noxious weed,
 - a plant or microorganism thought not to damage the ecosystem,
 - It should be noted that *possession* of DNA modified plants is covered in Section III-E-2 and Appendix P. Possession is not covered by the Guidelines for most other organisms. In all cases the act of generating recombinant DNA and its introduction to any organism is covered by the Guidelines.
 - Plants: Section III-E-2 deals with whole plants containing recombinant DNA or DNA modified organisms associated with plants. COMS requires committee approval for these organisms and their construction before the research starts. Detailed requirements are given in the Guidelines, Appendix P.

**“NO WAITING PLUS”
DON’T NOTIFY IBC
(From Section III-F)**

COMS DOES NOT RECOGNIZE THIS CATEGORY.

All recombinant DNA studies must be registered with and approved by COMS before they start.

- **EXEMPT RECOMBINANT DNA** (SECTION III-F AND APPENDIX C)
“Exempt” from NIH guidelines means that work with these constructs need not be approved by IBC.

SOME “EXEMPT” CLASSES ARE:

- DNA vaccines encoding epitopes from microbiological sources are generally exempt from the NIH Guidelines, even in human studies. This unusual exemption is found in Appendix VI-A.
- Recombinant DNA outside of living or viral organisms,
- Recombinant DNA that cannot replicate or express *in vivo*,
- DNA from a single nonchromosomal or viral source,
- The DNA source organism and the host organism are the same organism,
- The DNA source organism and the host organism normally exchange DNA (organisms that normally exchange are listed in Appendix A),
- The DNA that does “not present a significant risk to health or the environment...”,
- The recombinant DNA is used exclusively in tissue culture and has < ½ eukaryotic viral genome. *There are exceptions to this rule (Appendix C-I-A). Check with the Biosafety Office,*
- Experiments using an *E. coli* host vector system in which the host does not contain conjugation proficient plasmids. There are some restrictions on the vectors used (Appendix C-II). BL1 containment is suggested,
- Experiments with *Saccharomyces* host-vector systems. There are some restrictions (Appendix C-III). BL1 containment is suggested,
- Experiments with *Bacillus subtilis* or *licheniformis* host-vector systems and in which reversion to spore formation is < 10⁻⁷. There are some other restrictions (Appendix C-IV). BL1 containment is suggested.

THREE SPECIALIZED GUIDELINE SECTIONS

The NIH Guidelines recognize three non-laboratory classes of Biosafety containment and procedures; those in which genes are transferred into humans (Appendix M); those for plants (BL1-P through BL4-P, Appendix P) and those for animals (BL1-N through BL4-N, Appendix Q).

- **HUMANS**

All Human Gene Transfer protocols are currently considered experimental. IBC has established a Human Gene Therapy Advisory Committee to deal with human Gene Transfer studies.

IBC approval must await RAC (NIH Recombinant Advisory Committee) action. Depending on whether the study is deemed “novel” the RAC can either schedule a full examination of the protocol at one of its quarterly meetings or recommend sole FDA review.

Overcoming the regulatory hurdles involved in gaining approval for a human gene transfer study is not a task for the faint of heart. Beyond approvals from the Food and Drug Administration one has to get approval from the local Institutional Review Board, the Biosafety Committee. In addition the NIH Recombinant DNA Advisory Committee (the RAC) will evaluate novel protocols although it does not have approval power. These evaluations often involve the PI's appearance in Bethesda and aggressive questioning by members of the RAC.

For most people this process can be intimidating. Best to get help early either through a commercial sponsor or a consultant.

- **ANIMALS**

Animal Biosafety levels are normally used to cover large animals such as cattle, swine, horses, poultry . . . IBC tends to use the same designations when considering safe practices with lower animals including rodents.

All animal experiments must be reviewed and approved by a local Institutional Animal Care and Use Committee (IACUC). These committees act under US Department of Agriculture regulations.

- **PLANTS**

Plant Biosafety levels are necessary when research plants are too big, too many or have growth requirements that cannot be covered by the standard Biosafety Levels. The plant guidelines cover microorganisms and small “animals,” particularly insects, such as arthropods. Plant associated microorganism include viroids, virusoids, bacteria, viruses, fungi, protozoans, as well as benign or beneficial microorganisms known to be associated with plants (*Rhizobium*).

When studies covered under the plant appendix are being discussed IBC will include an expert in plant pests or containment.

It is of interest that the plant guidelines are not designed to directly protect humans from plant related recombinant DNA. The agents covered pose virtually no threat to humans or higher animals. Rather the guidelines are in place to protect the general ecosystem from serious disruption. Thus procedures are designed to limit the spread of novel organisms from the experimental facility, not to protect the workers.

HOW RISK GROUPS ARE TRANSLATED INTO BIOSAFETY LEVELS:

<ul style="list-style-type: none"> Any recombinant molecule that does not replicate in (Section III-B) 		
<ul style="list-style-type: none"> Gene Transfer into Human subjects (RAC review <u>process</u>² IS REQUIRED BEFORE IBC CAN APPROVE THIS KIND OF STUDY). (SECTION III-C-1). THERE ARE NO REGULATIONS AS TO CLINICAL CONTAINMENT. 		
<ul style="list-style-type: none"> RECOMBINANT ORGANISMS CULTURED IN VOLUMES GREATER THAN 10 LITERS (SECTION III-D-6). 		
<ul style="list-style-type: none"> RECOMBINANT VIRAL DNA SHORTER THAN 1/2 EUKARYOTIC VIRUS GENOME IS EXEMPT FROM THE GUIDELINES. (APPENDIX C-1) 		
<ul style="list-style-type: none"> PATHOGEN HOSTS (SECTION III-D-1): CHECK APPENDIX B TO DETERMINE THEIR RISK GROUP - THEN: 		
	RISK GROUP 2 HOST	BL2, BL2-N
	RISK GROUP 3 HOST	BL3, BL3-N
	RISK GROUP 4 HOST	BL4, BL4-N
<ul style="list-style-type: none"> PATHOGEN DNA SOURCE INTO NON-PATHOGEN HOST (SECTION III-D-2): CHECK APPENDIX B TO DETERMINE THEIR RISK GROUP - THEN: 		
	RISK GROUP 2 OR 3 SOURCE	BL2
	RISK GROUP 4 SOURCE	BL2, IF THE PATHOGEN GENOME IS DEFECTIVE
	RISK GROUP 4 SOURCE	BL4, OTHERWISE
<ul style="list-style-type: none"> ANIMAL VIRUS DNA SOURCE INTO TISSUE CULTURE (SECTION III-D-3): CHECK APPENDIX B TO DETERMINE THEIR RISK GROUP - THEN: 		
	RISK GROUP 2 VIRUS SOURCE	BL2
	RISK GROUP 3 VIRUS SOURCE	BL3
	RISK GROUP 4 VIRUS SOURCE	BL4
<ul style="list-style-type: none"> TRANSGENIC <i>ANIMAL</i>³ HOST (SECTION III-D-4): 		
	ANYTHING BUT > 2/3 EUKARYOTIC VIRUS GENOME	BL1-N (BUT IBC CAN BOOST THIS LEVEL BASED ON THE PATHOGENICITY OF THE SOURCE ORGANISM)
	VIRAL VECTORS THAT DON'T TRANSMIT	BL1-N
	EVERYTHING ELSE IS A SPECIAL CASE	IBC DECIDES
<ul style="list-style-type: none"> MODIFIED MICROORGANISMS INTO <i>ANIMALS</i>: (SECTION III-D-4) 		
	ANY VIABLE rDNA MODIFIED ORGANISM	≥BL2, BL2-N
<ul style="list-style-type: none"> rDNA INTO <i>ANIMALS</i> 		
	ANY rDNA THAT (EXCEPT PIECES >2/3 EUKARYOTIC VIRAL GENOME	BL1, BL1-N
	EVERY THING ELSE	IBC DECIDES
<ul style="list-style-type: none"> WHOLE <i>PLANTS</i>: (SECTION III-D-5) 		
	EXOTIC ⁴ PATHOGEN HOSTS THAT CAN DAMAGE THE ECOSYSTEM	BL3-P OR BL2-P+
	PLANTS WITH TRANSMISSIBLE DNA FROM EXOTIC PATHOGENS THAT CAN DAMAGE THE ECOSYSTEM	BL3-P OR BL2-P+, BL4-P
	TRANSMISSIBLE EXOTIC PATHOGEN HOSTS.....	BL4-P
	TOXIN DNA INTO PLANTS.....	BL3-P
	INSECT PATHOGEN DNA THAT CAN DAMAGE THE ECOSYSTEM.....	BL3-P OR BL2-P+
<ul style="list-style-type: none"> GENES CODING FOR VERTEBRATE TOXINS (APPENDIX F) 		
	LD ₅₀ < 100 NG/KG.....	REQUIRES NIH & IBC APPROVAL
	100 NG/KG < LD ₅₀ < 100 UG/KG	REQUIRES IBC APPROVAL & NIH NOTIFICATION. (EXCEPT IN E. COLI - SEE BELOW)
	100 NG/KG < LD ₅₀ < 1UG/KG	BL2 IF IN E. COLI
	1 UG/KG < LD ₅₀ < 100 MG/KG.....	BL1 IF IN E. COLI

²RAC can either take no action and transmit the protocol to the FDA or call for a full public review at one of its quarterly meetings.

³The purchase or transfer of transgenic rodents is exempt from the NIH guidelines.

⁴“Exotic” plant pathogens are defined as those not known to occur naturally in the US.

SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR INFECTIOUS AGENTS⁵

BSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard Microbiological Practices	None required	Open bench and sink required
2	<ul style="list-style-type: none"> • Agents associated with human disease • Routes of transmission include percutaneous injury, ingestion, mucous membrane exposure 	BSL-1 practice plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • “Sharps” precautions • Biosafety manual defining any needed waste decontamination or medical surveillance policies 	Primary barriers: <ul style="list-style-type: none"> • Class I or II BSCs or other physical containment devices used for all manipulations of agents that cause splashes or aerosols of infectious materials PPEs* : • Laboratory coats; gloves; face protection as needed 	BSL-1 plus: <ul style="list-style-type: none"> • Autoclave available
3	<ul style="list-style-type: none"> • Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious or lethal consequences 	BSL-2 practice plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of all waste • Decontamination of laboratory clothing before laundering • Baseline serum 	Primary barriers: <ul style="list-style-type: none"> • Class I or II BSCs or other physical containment devices used for all open manipulation of agents PPEs*: • Protective laboratory clothing; gloves; respiratory protection as needed 	BSL-2 plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double-door access • Exhaust air not recirculated • Negative airflow into laboratory
4	<ul style="list-style-type: none"> • Dangerous/exotic agents which pose high risk of life-threatening disease • Aerosol-transmitted laboratory infections have occurred; or related agents with unknown risk of transmission 	BSL-3 practices plus: <ul style="list-style-type: none"> • Clothing change before entering • Shower on exit • All material decontaminated on exit from facility 	Primary barriers: <ul style="list-style-type: none"> • All procedures conducted in Class III BSCs or Class I or II BSCs in combination with full-body, air-supplied, positive pressure personnel suit 	BSL-3 plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum, and decontamination systems • Other requirements outlined in the text

⁵ From Biosafety in Microbiological and Biomedical Laboratories, Fifth Edition, 2007.

SUMMARY OF RECOMMENDED BIOSAFETY LEVELS FOR ACTIVITIES IN WHICH EXPERIMENTALLY OR NATURALLY INFECTED VERTBRATE ANIMALS ARE USED.⁶

ABSL	AGENTS	PRACTICES	PRIMARY BARRIERS AND SAFETY EQUIPMENT	FACILITIES (SECONDARY BARRIERS)
1	Not known to consistently cause diseases in healthy adults	Standard animal care and management practices, including appropriate medical surveillance programs	As required for normal care of each species	Standard animal facility: <ul style="list-style-type: none"> • No recirculation of exhaust air • Directional air flow recommended • Hand washing sink is available
2	<ul style="list-style-type: none"> • Associated with human disease • Hazard: percutaneous exposure, ingestion, mucous membrane exposure. 	ABSL-1 practice plus: <ul style="list-style-type: none"> • Limited access • Biohazard warning signs • “Sharps” precautions • Biosafety manual • Decontamination of all infectious wastes and of animal cages prior to washing 	ABSL-1 equipment plus primary barriers: <ul style="list-style-type: none"> • Containment equipment appropriate for animal species PPEs* : • Laboratory coats, gloves, face and respiratory protection as needed 	ABSL-1 plus: <ul style="list-style-type: none"> • Autoclave available • Hand washing sink available • Mechanical cage washer recommended
3	<ul style="list-style-type: none"> • Indigenous or exotic agents with potential for aerosol transmission • Disease may have serious health effects 	ABSL-2 practice plus: <ul style="list-style-type: none"> • Controlled access • Decontamination of clothing before laundering • Cages decontaminated before bedding removed • Disinfectant foot bath as needed 	ABSL-2 equipment plus: <ul style="list-style-type: none"> • Containment equipment for housing animals and cage dumping activities • Class I, II or III BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: • Appropriate respiratory protection 	ABSL-2 facility plus: <ul style="list-style-type: none"> • Physical separation from access corridors • Self-closing, double-door access • Sealed penetrations • Sealed windows • Autoclave available in facility
4	<ul style="list-style-type: none"> • Dangerous/exotic agents that pose high risk of life threatening disease • Aerosol transmission, or related agents with unknown risk of transmission 	ABSL-3 practices plus: <ul style="list-style-type: none"> • Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting • All wastes are decontaminated before removal from the facility 	ABSL-3 equipment plus: <ul style="list-style-type: none"> • Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities 	ABSL-3 facility plus: <ul style="list-style-type: none"> • Separate building or isolated zone • Dedicated supply and exhaust, vacuum and decontamination systems • Other requirements outlined in the text

PPE – Personal Protective Equipmen

⁶ From Biosafety in Microbiological and Biomedical Laboratories, Fifth Edition, 2007.