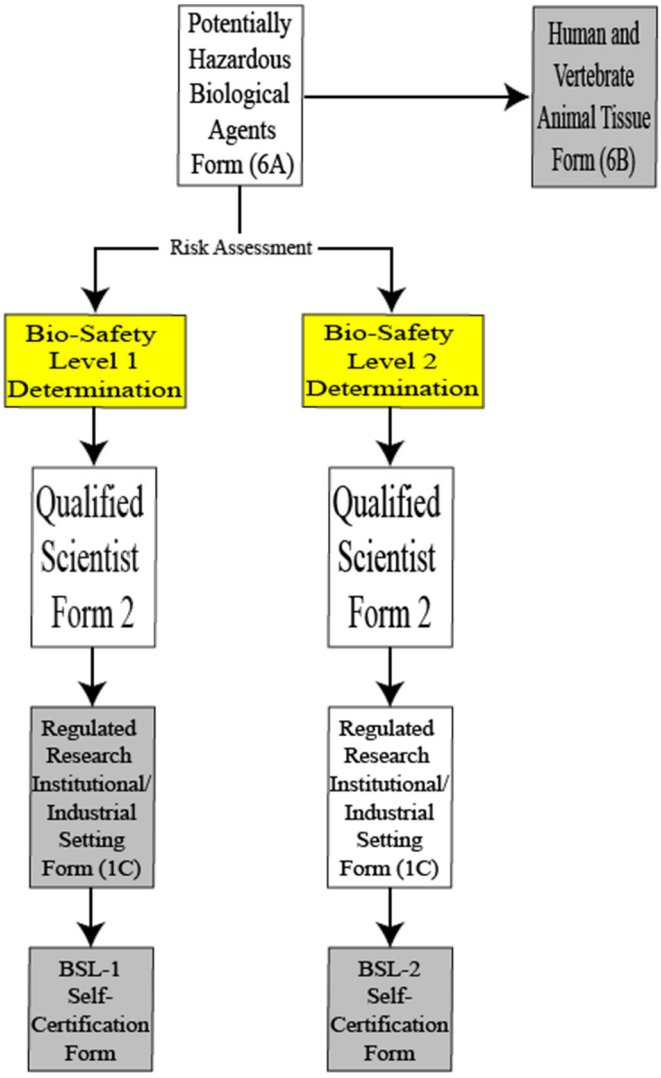
Potentially Hazardous Biological Agents Rules

Research using microorganisms (including bacteria, viruses, viroids, prions, rickettsia, fungi, parasites), recombinant DNA technologies or human or animal fresh/frozen tissue, blood, or bodily fluids may involve potentially hazardous biological agents.

When dealing with potentially hazardous biological agents, it is the responsibility of the Student Researcher(s) and ALL of the adults involved in a research project to conduct and document a risk assessment (Form 6A on page 33) to define the potential level of harm, injury or disease to PLANTS, ANIMALS and HUMANS that may occur when working with biological agents.

The risk assessment determines the biosafety level, which in turn determines if the project can proceed, and if so, the laboratory facilities, equipment, training and supervision required.



All projects involving potentially hazardous biological agents must be reviewed and approved BEFORE experimentation begins by the appropriate review board: IBC (for studies done at a research institution) or SRC (for studies done in a school setting).

- Experimentation involving the culturing of any organism (even BSL-1) is **PROHIBITED in a home environment**. Specimens may be collected at home or other field sites as long as they are immediately transported to a laboratory with the appropriate BSL containment as determined by the school/local SRC. *Specimen collection sites must be de-identified on the project board (use labels like Site A, B, etc.)*
- The initial risk assessment determination done by the Student Researcher/Team Leader and Qualified Scientist/Mentor must be confirmed by the appropriate review board.
- Student Researchers must be trained in standard microbiological practices.
- Once the study has been approved, if the Student Researcher has any proposed changes to the methods and/or procedures, they must repeat the review process before continuing with data collection/ experimentation.
- ALL PHBAs must be properly disposed of, by the Designated Supervisor or Qualified Scientist/Mentor, at the end of experimentation in accordance with their biosafety level. Acceptable disposal methods for BSL-1 and BSL-2 organisms include:
  - Autoclave at 121°C for 20 minutes;
  - Use of a 10% bleach solution (1:10 dilution of domestic bleach);
  - Incineration;
  - Alkaline hydrolysis;
  - Biosafety pick-up; or
  - Other manufacturer recommendations.

Potentially Hazardous Biological Agent Study Biosafety Levels (BSL)

- **BSL-1** - biological agents that pose low risk to personnel and the environment; highly unlikely to cause disease in healthy laboratory workers, animals or plants
  - BSL-1 research projects must be conducted in a BSL-1 or higher laboratory. This MAY be a middle or high school science lab if it meets ALL of the standards for a BSL-1 lab (see the self-certification form at http://www.csef.colostate.edu/Guidelines/Guidelines_BSL1.pdf).
  - BSL-1 research projects must be reviewed by a Qualified Scientist/Mentor, but can be directly supervised by a TRAINED Designated Supervisor at a verifiable BSL-1 laboratory.
  - Examples of BSL-1 Organisms: *Agrobacterium tumefaciens* (soil bacteria), *Micrococcus luteus*, *Neurospora crassa* (red bread mold), *Bacillus subtilis* (normal human gut bacteria).
  - Examples of BSL-1 Studies (this is not an exhaustive list):
    - Studies involving naturally-occurring plant pathogens where they are not cultured or introduced into the environment.
    - rDNA technology studies involving BSL-1 organisms and BSL-1 host vector systems (i.e.: cloning of DNA in *E. coli K-12*, *S. cerevisiae*, and *B. subtilis* host vector systems).
    - Studies involving commercially available rDNA technology kits using BSL-1 organisms.
    - Studies of mold growth on food items where the project is NOT terminated at the first sign of mold.
    - Studies involving unknown microorganisms collected from the environment as long as **ALL of the following conditions are followed**:
      - Culturing is done in a plastic Petri dish and is **SEALED**.
      - The Petri dish remains **SEALED** throughout the experimentation.
      - The **SEALED** Petri dish is disposed of via autoclaving or disinfection by the Designated Supervisor or Qualified Scientist/Mentor.
    - Studies involving genome editing with possible biological impact, including alteration of germline cells.
    - Studies that insert antibiotic resistant markers for the clonal selection of bioengineered organisms.

- **BSL-2** - biological agents that pose moderate risk to personnel and the environment; exposure in a lab situation would result in limited risk of spreading and it would rarely cause infection that would lead to serious disease; in the event that infection occurs, treatment and preventive measures are available
  - BSL-2 research projects must be conducted in a BSL-2 or higher laboratory. This is usually a regulated research institution, but a high school science lab MAY QUALIFY if it meets ALL of the standards for a BSL-2 lab (see the self-certification form at http://www.csef.colostate.edu/Guidelines/Guidelines_BSL2.pdf).
  - BSL-2 research projects must be reviewed and directly supervised by a Qualified Scientist/Mentor at a verifiable BSL-2 laboratory.
  - Examples of BSL-2 Organisms: *Mycobacterium* (typically found in water and food sources), *Steptococcus pneumoniae* (part of the normal upper respiratory tract flora), *Salmonella choleraesuis* (typically found in raw food sources such as eggs and meat).
  - Examples of BSL-2 Studies (this is not an exhaustive list):
    - Studies culturing known MRSA, , VISA/VRSA, VRE, CRE and ESBL can only be done at a Regulated Research Institution and must include written justification for their usage with documented IBC review and approval.
    - Studies that select and subculture antibiotic-resistant organisms. Use **EXTREME CAUTION** when doing this type of project.
    - Studies that culture human or animal waste (including sewage sludge).
    - Studies that insert antibiotic resistant markers for the clonal selection of bioengineered organisms.

- rDNA technology studies using BSL-1 agents that may convert to BSL-2 agents during the course of experimentation.
- rDNA technology studies involving BSL-2 organisms and/or BSL-2 host vector systems.
- Studies involving unknown organisms collected from the environment where the culturing container (Petri dish) is opened for any purpose (except for disposal disinfection).

- **BSL-3** – biological agents that usually cause serious disease (human, animal or plant) or that can result in serious economic consequences
- **BSL-4** – biological agents that usually produce very serious disease (human, animal or plant) that is often untreatable

Prohibited PHBA Studies:

- Genetically engineered organisms with multiple drug resistance traits with the intended purpose of investigating the pathology or treatment of antibiotic-resistant infections.
- Insertion of antibiotic-resistant traits or selection of organisms expressing traits that may affect the ability to provide effective treatment of infections acquired by humans, animals or plants.
- BSL-3 AND BSL-4 research projects.
- Propagation of recombinant containing DNA coding for human, plant or animal toxins (including viruses).
- The introduction or disposal of non-native, genetically-altered and/or invasive species, pathogens, toxic chemicals or foreign substances into the environment.

Studies Exempt from Prior SRC Review/Approval

The following types of studies are exempt from prior SRC review and approval, but **MUST** be included on the Risk Assessment Form 3.

- Studies involving baker's yeast and brewer's yeast, except in rDNA studies.
- Studies involving *Lactobacillus* (starter cultures for controlled fermentation), *Bacillus thurgensis* (typically found in insecticides), nitrogen-fixing/oil-eating bacteria, and algae-eating bacteria introduced into their NATURAL ENVIRONMENT. **None of these studies are exempt if they are cultured in a Petri dish.**
- Studies involving water or soil where the Student Researcher(s) is not purposely culturing bacteria.
- Studies of mold growth on food items, **IF the experiment is TERMINATED at the first sign of mold.**
- Studies of edible mushrooms and slime molds.
- Studies involving *E. coli k-12* which are done at school and are not rDNA studies.
- Studies involving protists or archaea.
- Studies using manure for composting, fuel production or other non-culturing experiments.
- Studies involving the use of commercially-available color change coliform water test kits. These kits must remain sealed and be properly disposed.
- Studies involving the decomposition of vertebrate organisms (such as in forensic projects).
- Studies with microbial fuel cells. The device must remain sealed and be properly disposed.