Potentially Hazardous Biological Agents

image from www.lifesciencesindex.com

Revised August 2016

CREST-Jane Goodall Science Symposium

Supplemental

Guidelines & Requirements

for your

ISEF Research Plan

When doing microbiological research (including bacteria, viruses, viroids, prions, rickettsia, fungi, and parasites), recombinant DNA technologies, or human/animal tissues or cells (fresh or frozen), blood or body fluids, you are working with **potentially hazardous biological agents (PHBAs)**. You will need to follow all the local, state and federal rules and guidelines that apply to university-level studies. **Your research plan must include information that shows how you will follow those guidelines.** A special committee will review your plan, determine its risk level, and approve your project before you begin experimenting. This committee is called the Scientific Review Committee, or SRC, and includes teachers and professional scientists who have volunteered to review student projects to make sure no harmful effects come from your research.

In this packet, you'll find:

1. A list of extra requirements that must be included in your research plan, from the Society for Science and the Public's ISEF rulebook.
2. An explanation of how to figure out the risk level for your project, from the ISEF rulebook. Any research that is determined by the SRC to be greater than we can safely house at the high school (BSL-2 or above) must be conducted at a university or other research institution.
3. An example laboratory protocol to help guide you in writing your own.

CHECKLIST: Requirements for ISEF research plans involving PHBAs

adapted from: https://member.societyforscience.org/document.doc?id=642

The following items must be covered in the procedure and risk assessment sections of your research plan. BE SURE you have included each item in this list before you submit your plan for review!:

- ☐ Procedure: discuss media and microbes you will use, and how many samples you'll test, what incubation method you'll use, where you will do this study, how you will collect data
- ☐ Procedure: Give source of agent, source of specific cell line, etc. (Where are you getting your PHBA? Is it a specific strain? Who will order it or collect it and what form will it come in?)
- ☐ Risk Assessment: Describe Biosafety Level Assessment process and resultant BSL determination (see the section below for more information about this).
- ☐ Risk Assessment: Detail safety precautions (You will be trained in basic microbiological lab safety practices, and you can also find tutorials online which will help you understand what safety precautions should be followed when in the lab and when you start and finish experimenting).
- ☐ Risk Assessment: Discuss methods of disposal (All potentially hazardous biological agents must be properly disposed at the end of experimentation in accordance with their biosafety level. For BSL 1 or BSL 2 organisms: Autoclave at 121 degrees Celsius for 20 minutes, OR use of a 10% bleach solution (1:10 dilution of domestic bleach) are acceptable).

How do you write the Risk Assessment for your project?

https://student.societyforscience.org/Potentially-Hazardous-Biological-Agents

Use this information to understand the risk level of your project. This will help you write your Risk Assessment section in your research plan.

Potentially Hazardous Biological Agents Risk Assessment

Risk assessment defines the potential level of harm, injury or disease to plants, animals and humans that may occur when working with biological agents. The end result of a risk assessment is the assignment of a biosafety level which then determines the laboratory facilities, equipment, training, and supervision required.

Risk assessment involves:

- Assignment of the biological agent to a risk group (Note from school: search this online)
- Studies involving a known microorganism must begin with an initial assignment of the microorganism to a biosafety level risk group based on information available through a literature search.
- The study of unknown microorganisms and the use of fresh tissues relies on the expertise of the supervising adult(s).
- Determination of the level of biological containment available to the student researcher to conduct the experimentation. (See "Levels of Biological Containment" for details.)
- Assessment of the experience and expertise of the adult(s) supervising the student.
- Assignment of a biosafety level for the study based on risk group of biological agent, level of biological containment available and the expertise of the Qualified Scientist or Designated Supervisor who will be supervising the project.

Documentation of review and approval of study prior to experimentation:

- If a study is conducted at a non-regulated site (e.g. school), the SRC reviews the Research Plan.
- If the study was conducted at a Regulated Research Institution, and was approved by the appropriate institutional board (e.g. IBC, IACUC), the SRC reviews the institutional forms provided and documents SRC approval (Form (6A)).
- If a PHBA study was conducted at a Regulated Research Institution but the institution does not require review for this type of study, a letter from an institutional representative stating that review was not required must be obtained. The SRC must review the study and document approval on Form 6A that the student received appropriate training and the project complies with Intel ISEF rules.

Classification of Biological Agents

Risk Groups

Biological agents, plant or animal, are classified according to biosafety level risk groups. These classifications presume ordinary circumstances in the research laboratory, or growth of agents in small volumes for diagnostic and experimental purposes.

BSL-1 risk group contains biological agents that pose low risk to personnel and the environment. These agents are highly unlikely to cause disease in healthy laboratory workers, animals or plants. The agents require Biosafety Level 1 containment. Examples of BSL-1 organisms are: *Agrobacterium tumifaciens*, *Micrococcus leuteus*, *Neurospora crassa*, *Bacillus subtilis*.

BSL-2 risk group contains biological agents that pose moderate risk to personnel and the environment. If exposure occurs in a laboratory situation, the risk of spread is limited and it rarely would cause infection that would lead to serious disease. Effective treatment and preventive measures are available in the event that an infection occurs. The agents require Biosafety Level 2 containment. Examples of BSL-2 organisms are: Mycobacterium, Streptococcus pneumonia, Salmonella choleraesuis.

BSL-3 risk group contains biological agents that usually cause serious disease (human, animal or plant) or that can result in serious economic consequences. Projects in the BSL-3 group are prohibited.

BSL-4 risk group contains biological agents that usually produce very serious disease (human, animal or plant) that is often untreatable. Projects in the BSL-4 group are prohibited.

Levels of Biological Containment

There are four levels of biological containment (Biosafety Level 1–4). Each level has guidelines for laboratory facilities, safety equipment and laboratory practices and techniques.

BSL-1 containment is normally found in water-testing laboratories, in high schools, and in colleges teaching introductory microbiology classes. Work is done on an open bench or in a fume hood. Standard microbiological practices are used when working in the laboratory. Decontamination can be achieved by treating with chemical disinfectants or by steam autoclaving. Lab coats and gloves are required. The laboratory work is supervised by an individual with general training in microbiology or a related science.

BSL-2 containment is designed to maximize safety when working with agents of moderate risk to humans and the environment. Access to the laboratory is restricted. Biological safety cabinets (Class 2, type A, BSC) must

be available. An autoclave should be readily available for decontaminating waste materials. Lab coats and gloves are required; eye protection and face shields must also be worn as needed. The laboratory work must be supervised by a scientist who understands the risk associated with working with the agents involved.

BSL-3 containment is required for infectious agents that may cause serious or potentially lethal diseases as a result of exposure by inhalation. Projects in the BSL-3 group are prohibited.

BSL-4 containment is required for dangerous/exotic agents that pose high risk of life-threatening disease. Projects in the BSL-4 group are prohibited.

Sources: Potentially Hazardous Biological Agents

1) American Biological Safety Association: ABSA Risk Group Classification – list of organisms
www.absa.org
2) American Type Culture Collection (ATCC)
www.atcc.org
3) Bergey's Manual of Systematic Bacteriology website – follow the links for resources and microbial databases for a collection of international websites of microorganisms and cell cultures: www.bergeys.org/resources.html
4) Biosafety in Microbiological and Biomedical Laboratories (BMBL) – 4th Edition. Published by CDC-NIH,
www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
5) World Health Organization Laboratory Safety Manual
www.who.int/diagnostics_laboratory/guidance/en/
6) Canada – Agency of Public Health – list of non-pathogenic organisms
www.phac-aspc.gc.ca/lab-bio/index_eng.php
www.phac-aspc.gc.ca/lab-bio/res/index-eng.php
7) Microorganisms for Education Website – list of organisms
www.science-projects.com/safemicrobes.htm
8) NIH Guidelines for Research Involving Recombinant DNA Molecules. Published by National Institutes of Health.
http://oba.od.nih.gov/oba/index.html
9) OSHA – Occupational Health and Safety Administration
www.osha.gov

Using Common Protocols

The following is an example of a laboratory protocol that can help you write your own in your Research Plan. You will not need to detail out every single step for any process with a common/standard protocol. You do, however, need to know every detail so that you are able to carry out the protocol in the laboratory and these details should be noted in your logbook.

BASIC Example –Quantifying bacterial growth with known/potential antibiotics

1. Make nutrient broth media

2. Inoculate each 25ml tube with 20uL of E. coli (strain K12, obtained from Carolina Biological Supply Co, 1 vial freeze-dried, started according to standard protocol, BSL-1).

3. Subculture E. coli, 20uL each into 30 tubes per treatment, three treatments (amoxicillin, caffeine, vanilla .1M) after incubation at 37degC for 24 hours

4. After 72 hours, subculture to nutrient agar plates (30 per treatment) and take 1000 uL for spectrophotometry (index of cell counts based on light absorbance).

5. Quantify growth by absorbance at 540 nm at 72 hours incubation, and CFU count at 96 hours.

←*Note*: there are many detailed steps to making media, including measuring out ingredients and amendments, autoclaving the material, pouring the media into plates, etc., but as making media is an established protocol, no further details about it will need to be explained in your research plan. Put that detail in YOUR LOGBOOK. The rest of the steps in this protocol also follow that rule.

Some important reminders on laboratory safety that should be noted in your risk assessment

- No food or drink in the laboratory and other ways to avoid ingestion of poisonous or toxic materials.
- Importance of wear gloves and goggles when working with microorganism, chemicals, and other risk agents
- Know the location of all safety and emergency equipment (eye wash, first aid kit, fire extinguisher, etc.)
- Clean and disinfect your work area before and after your experimentation. This also includes properly washing your hands.
- Identify safety rules when using sharp objects like scalpels. Note in your risk assessment ways to reduce cuts by always pointing tips down and away from your body, cutting away from your body, and never trying to catch a falling sharp object.
- Cite the use of proper laboratory dress code- This is usually long pants, closed-toe shoes, long hair pulled back, and often the use of a laboratory coat.