Los Angeles County Science & Engineering Fair
Inspiring Student Discovery & Innovation
1107 Fair Oaks Ave. # 94, South Pasadena, CA 91030 www.lascifair.org

*This is a **fillable PDF**. Please **download** this document to your device and enter your responses in the given boxes. You do not need to complete the form in one sitting. Choose Save to save your progress. Once you complete the form, get it approved by your adult supervisor and site coordinator BEFORE you go online to complete the online pre-approval form. You can then copy and paste your responses in the appropriate sections of the online form.*

Research Plan for Experiments with Microbes

GUIDELINES FOR MICROBIAL RESEARCH AND SAFETY PRECAUTIONS

Students planning research involving the use of microbes must complete and obtain LACSEF Scientific Review Committee (SRC) approval of certification before starting experiments. Projects will not be accepted in the annual science and engineering fair without approval.

The following are examples of precautions that must be taken to prevent injury to persons or the environment. No list could possibly foresee all possible hazards, so teachers, parents and students must carefully plan and follow safe procedures specific to each study. The methods and materials section of the project description must contain explicit and detailed statements as to how and where experiments will be conducted.

1. All cultures in Petri dishes must be bound together with transparent tape, immediately after exposure/inoculation. Any Petri dish that contains fungus/mold should be taped shut all the way around the edges. Examine through lids only.
2. All bacteria, protozoa and fungi (including molds) are to be handled as though pathogenic (disease-causing). Bacteria KNOWN to be pathogenic are not to be cultured. **Pure cultures of non-pathogenic microorganisms should be used in experiments.** Laboratory studies utilizing MRSA (Methicillin-resistant Staphylococcus aureus) and VRE (VancomycinResistant Enterococcus) are prohibited.
3. **Environmental Sampling of unknown microorganisms from school grounds, household surfaces and field sites off campus.** - These studies present a challenge because the identity, concentration, and pathogenicity of the cultured agents are unknown. Unknown microorganisms should be collected with proper safety procedures, samples sealed immediately and cultured in a school or institutional laboratory, NOT at home. When soil or water is used as a source of bacteria (or fungi), it is important to collect samples unlikely to be contaminated by human pathogens and never from areas suspected to be or posted as polluted. Collection of soil samples in or around old building sites, animal burrows and/or areas in which valley fever is endemic should be avoided.
4. Bacterial studies must be conducted in a properly equipped school or institutional laboratory under qualified adult supervision. No experimentation using existing antibiotic-

resistant microorganisms may be conducted at home or at school. Students may NOT be directly involved in the acquisition of microbes (exception: microbe collection in the environment using sterile swabs and appropriate collection techniques and supervision.

5. Inoculating loops must be used with care. Wire loops used for transferring bacteria cultures should be flamed until the entire wire is red hot before and after each transfer is made. Petri dishes that are inoculated with materials containing unknown microorganisms (i.e the material might not be a pure non-pathogenic culture) must not contain blood agar or Brain Heart Infusion (BHI) Broth but rather nutrient or trypticase soy agar.
6. These safety precautions are intended for experimental activities involving any bacteria or fungi. Even nonpathogenic microbes may cause disease if they enter the body accidentally. Autoclave or disinfect all waste material; disinfect work areas with 10% bleach, use gloves and goggles.
    a. **Glass Petri dishes:** to sterilize plates before cleaning or disposal, follow these steps:
        i. Autoclave the unopened plates in the usual manner. Usually, steaming at a pressure of 15 pounds per square inch for 15 to 20 minutes kills most microbes. However, to sterilize soil samples or large volumes of culture, continue with the procedure described below.
        ii. Wait one day for any resistant spores to leave the resting stage and begin to grow, sterilize a second time.
        iii. Wait one more day, sterilize a third time - discard sterilized cultures in the regular trash.
    b. **Disposable plastic petri dishes:** place unopened, sealed dishes in Biohazard disposal bags (included in Science Supply kits for E.coli and molds) and use District pick-up of bags as hazardous waste. Calls to nearby universities and hospitals can also yield a place to dispose of microbial waste.
    c. **Sterilizing plates of pure, non-pathogenic bacterial cultures**: the materials can be covered with a 10% bleach solution and allowed to soak for at least 1 to 2 hours. Discard sterilized cultures in the regular trash.
7. Experimentation with molds or other fungi must take place in a fume hood or open-air area (to prevent contamination of living areas with fungal spores or exposure to allergens). If anyone in the area has a damaged immune system or any allergies, experiments with molds/fungi must be conducted in a laboratory. Containers must be sealed airtight at all times during observations and disposed of as possible pathogens.
8. Research involving pathogenic or potentially pathogenic agents shall be conducted following standard microbiological practices as defined in Biosafety in Microbiological and Biomedical Laboratories (BMBL) published by CDC-NIH. All projects must conform to the CA Education Code Title 2, Division 2, Part 28, Chapter 4, Article 5, 51540.
9. Arrangements must be made to assure that any proposed procedure is safe before any project proposal is approved. Whenever specialized safety equipment and/or facilities (e.g., fume hoods, clinical laboratory) are necessary for a procedure, arrangements must be made in advance. Please contact the LACSEF SRC for questions or assistance at Pre-approval@lascifair.org

Student Name	
School	
Email (non-school)	

☐ I certify that I have read and understand the guidelines for vertebrate research and safety precautions as outlined in the LACSEF Rules and Regulations (check box)

In addition to this plan, I have also completed the following research plan(s) for this project (check all that apply).

☐ Hazardous Material

☐ Tissue, Cell Lines, Organs or Organ Parts

☐ Human Subjects

☐ Vertebrates

☐ No other research plan was submitted

Title:

- Title must be limited to150 characters (including spaces)

Problem

- In the form of a question

Objective(s)

- Your goal for the project - why is it important

Hypothesis

- (Example “IF I do this… Then this will happen”

Number of Participating Students

- This refers to the number of students conducting the project, not the number of test subjects.
- There are a maximum number of three students allowed on a project team.

Microbe Description and Source

Microbe Type

- Describe the type of microbes (bacteria, molds, fungus, viruses, and/or protozoans) involved in your experiment and their species name, if known.
- Microbes are defined as pathogenic or potentially pathogenic agents including bacteria, viruses, viroids, prions, Rickettsia, fungi, or parasites. Make sure you identify the genus and species name, where possible. Bacteria KNOWN to be pathogenic are not to be cultured. **Pure cultures of non-pathogenic microorganisms should be used in experiments.** Laboratory studies utilizing MRSA (Methicillin-resistant Staphylococcus aureus) and VRE (Vancomycin-Resistant Enterococcus) **<u>are prohibited</u>**.

Collection Location

- Describe where the bacteria, protozoa, fungi, molds, etc. will be collected AND the location of culturing. Give the specific address where the majority of the project will take place; both sampling and experimentation. **Environmental sampling of unknown microorganisms** from school ground,household surfaces and field sites off campus poses a challenge because the identity, concentration, and pathogenicity of the cultured agents are unknown. Unknown organisms should be collected with proper safety procedures, samples sealed immediately, and cultured in a school or institutional laboratory, **NOT** at home.
- **No experimentation using existing antibiotic-resistant microorganisms may be conducted at home or at school. Exception: microbe collection in the environment using sterile swabs and appropriate collection techniques and supervision.**
- When soil or water is used as a source of bacteria (or fungi), it is important to collect samples unlikely to be contaminated by human pathogens and never from areas suspected to be or posted as polluted. Collection of soil samples in or around old building sites, animal burrows, and/or areas in which valley fever is endemic should be avoided.

Microbe Source

- Describe the source for the microbe (Full detail required).
- Students may NOT be directly involved in the acquisition of microbes. Exception: microbe collection in the environment using sterile swabs and appropriate collection techniques and supervision. When microbes are obtained by the student from an institution or Biomedical Scientist, an email verification by the scientist will need to be completed BEFORE the SRC/pre-approval process can begin.

Procedures

Student Procedure

- Describe the procedures to be performed by the student.
- List the step-by-step procedures that the student alone will perform. The student and Designated Adult Supervisor may consult with a Biomedical Scientist to obtain detailed instructions and guidance in the techniques to be used by the student under the direct continuous supervision of the Designated Adult Supervisor (for research not conducted in the Biomedical Scientist's laboratory).
- In culturing microbes, inoculating loops must be used with care. Wire loops used for transferring bacteria cultures should be flamed until the entire wire is red hot before and after each transfer is made.

Supervisor Procedures

- Describe the procedures to be performed by the supervising scientist/adult supervisor.

Culture Medium

- Identify what culture medium will be used.

Petri Dish Procedures

- If your experiment does not require the use of Petri dishes, state “My project does not require the use of petri dishes.”
- Describe the method and timing of sealing petri dishes.
- All cultures in Petri dishes must be bound together with transparent tape immediately after exposure/inoculation. Any Petri dish that contains fungus/mold should be taped shut. Examine through lids only.

Disposal Methods

1. Describe the disposal method(s) to be used for any hazardous materials, in detail.
2. Autoclave or disinfect all waste material; disinfect work area with 10% bleach, use gloves and goggles,.
3. **Glass Petri dishes -** To sterilize plates before cleaning for disposal follow these steps;
4. Autoclave the unopened plates in the usual manner. Usually, steaming at a pressure of 15 pounds per square inch for 15 to 20 minutes kills most microbes. However, to sterilize samples or large volumes of culture, continue with the procedure described below.
5. Wait one day for any resistant spores to leave the resting stage and begin to grow, sterilize a second time.
6. Wait one more day, sterilize a third time - discard sterilized cultures in the regular trash.

- **Disposable plastic petri dishes:** - place unopened, delayed dishes in Biohazard disposal bags (included in Science Supply kits), for E. coli and mold(s) use District pick-up bags as hazardous waste. Calls to nearby universities and hospitals can also yield a place for microbial waste.
- **Sterilizing plates of pure, non-pathogenic bacterial cultures**: - the materials can be covered with a 10% bleach solution and allowed to soak for at least 1 to 2 hours. Discard sterilized cultures in the regular trash.
- **Containers with mold** must be sealed airtight at all times during observations and disposed of as possible pathogens.

Safety Precautions

- See "Guidelines For Microbial Research And Safety Precautions"
- Arrangements must be made to assure that any proposed procedure is safe before any project proposal is approved. Even nonpathogenic microbes may cause disease if they enter the body accidently. Disinfect work area with 10% bleach, gloves and goggles. Whenever specialized safety equipment and/or facilities (e.g. fume hoods, clinical laboratory) are necessary for procedure, arrangements must be made in advance. If conducted at an institutional setting like a college or hospital, how will you follow standard microbial practices as defined in Biosafety in Microbial and Biomedical Laboratories (BMBL), published by CDV-NH.
- Bacterial studies must be conducted in a properly equipped school or institutional laboratory under qualified adult supervision.
- Experimentation with molds must take place in a fume hood or open-air (to prevent contamination of living areas with fungal spores). If anyone in the area has a damaged immune system or any allergies, experiments with molds must be conducted in a laboratory.

COVID-19 Risks

- Due to the special circumstances brought on by the COVID-19 pandemic, it is strongly recommended that ALL students include in their risk assessment how they will mitigate the spread of the disease while conducting their experiment. Such mitigations may be found at: https://www.societyforscience.org/isef/covid-policy/

Bibliographic References

- Provide bibliographic references for your project.
- References should be written in APA format
- At least one reference must be from a source other than the internet.
- Junior Division projects require at least three references.
- Senior Division projects require five references.

Reference 1

Reference 2

Reference 3

Reference 4

Reference 5

Certification References

Please provide the email addresses for the people who will be serving in the following roles in your experiment. An email will be sent to each address with a link for the person to certify your project. You can see what qualifications each person needs on our website.

Teacher/Advisor	
Name	
Email Address	
Qualifications	

Biomedical Scientist	
Name	
Email Address	
Qualifications	

Microbe Provider	
Name	
Email Address	

Designated Adult Supervisor	
Name	
Email Address	
Qualifications	

☐ By checking this box, I certify that the experimental procedures used in this project follow the rules and regulations of the LACSEF. I also certify that the procedure followed will ensure that neither the procedures nor the materials constitute any known danger and that all microorganisms, pathogenic or non-pathogenic, will be handled and disposed of as if pathogenic. I understand that this form must be approved and signed by all parties BEFORE the project can begin, and I will comply with all regulations.

If your project involves humans in any way, you need to complete the **Human Subjects Form at this Link:**