Mock Research Review Exercise

Exercise Scenarios

Training Goal

The mock review exercise uses team-based learning and role-playing approaches to train scientists to identify and analyze risks and benefits of a research project. This exercise does not involve the use of a reference list of high-risk experiments or research to assist with the reviews. Instead, this exercise is designed to promote critical thinking about biosafety and biosecurity risks associated with research, benefits of research, and strategies for mitigating risks and maximizing benefits.

Lead Developing Organization

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License

Notional Research Project 1

Project Title: Development and Testing of New Avian Influenza (H5N1) Virus Vaccines

Brief Summary: Researchers at the University of X have requested approval for a research proposal to conduct projects related to the development and testing of H5N1 vaccines. The researchers propose to construct a recombinant version of an H1N1 vaccine that has previously been approved for research use. They propose to modify this construct to include vaccine candidates against H5 variants and test them in chickens to determine effectiveness against virulent H5N1 virus. The researchers also propose to develop a novel adjuvant that they hope will increase the efficacy of candidate vaccines. The adjuvant, AVx, is a potential nasal adjuvant. The researchers expect that AVx-adjuvanted vaccines will demonstrate H5N1-specific cell-mediated immunity that will result in complete protection of homologous H5N1 virus challenge, as demonstrated in mice. Specifically, their research proposal includes the use of non-lethal and lethal H5N1 viral dose challenges after administration of the AVx-adjuvanted vaccine to mice. Approximately 150 mice will be used over a 12-week study. Live H5N1 virus will be used. After treatment with AVx-adjuvanted vaccines and dosing with H5N1 virus, mice will be sacrificed and antibody-secreting cells will be examined.

Project Goal: The goal of these studies is to develop a more effective and robust vaccine to prevent H5N1 influenza virus infection in poultry, which is a significant agricultural problem in the researchers' region.

Specific Aim 1: The researchers seek to develop the experimental vaccine of recombinant H5N1 viruses. To address this Aim, the researchers create a recombinant H1N1 vector and modify the vector by replacing the H1 gene with different variations of the H5 gene.

Specific Aim 2: The researchers seek to test the efficacy of the experimental recombinant H5N1 vaccines *in vivo*. To address this Aim, the researchers administer chickens with the experimental vaccines intranasally and intradermally. After vaccination, the researchers administer lethal and non-lethal doses of natural H5N1 virus or a control virus using spray delivery in chickens. At various times after challenge, the researchers evaluate the physical properties of the challenge and control groups, and collect blood and tissue samples to determine virus levels, cell-mediated immunity, levels of neutralizing antibodies, and markers on proliferated B and T cells.

Specific Aim 3: The researchers seek to test the efficacy of the AVx-adjuvanted experimental recombinant H5N1 vector *in vivo*. To address this Aim, the researchers jointly administer mice with the AVx-adjuvant and the experimental vaccines intranasally or intradermally. After vaccination, the researchers administer lethal and non-lethal doses of natural H5N1 viruses or a control virus intranasally in mice. At various times after challenge, the researchers evaluate the physical properties (e.g., weight) of the challenge and control groups, and collect blood and tissue samples to determine virus levels, cell-mediated immunity, and levels of neutralizing antibodies.

Notional Research Project 2

Project Title: Detection and Monitoring of Aflatoxin B1 in Chickens Sold in Live Poultry Markets

Brief Summary: Preliminary evidence in the meat processing industry near the University of X suggests that chicken products (e.g., livers, gizzards) are reservoirs for aflatoxins and toxin residues that can decrease economic value in the poultry industry. Researchers at the University of X have requested approval for a research proposal to conduct surveillance and chicken collection at local markets and points of export. The team proposes to bring live chickens back to the laboratory, where they will make a benchtop necropsy unit within a laminar airflow cabinet. Livers and other chicken tissues will be collected and assayed for Aflatoxin B1. The researchers also are proposing the development of a new ELISA assay for more accurate quantification of AFB1 levels that can be used at sites of meat production.

Project Goal: The goal of the study is to identify the prevalence of Aflatoxin B1 in live poultry markets, which can lead to the sale of contaminated meat and reduction in people purchasing poultry.

Specific Aim 1: The researchers develop a new ELISA method for detecting and quantifying Aflatoxin B1 levels in the tissue samples. To achieve this Aim, the researchers obtain tissue samples from chickens bred as laboratory animals. The researchers homogenize the tissues in different buffers to determine the level of background reactivity to a rabbit secondary antibody, which they purchased from a biotechnology company, using a Western Blot assay. They test the reactivity of the homogenized samples to a rabbit polyclonal Aflatoxin B1 antibody, which they make and purify in their laboratory, using a dilution series method and Western Blot assay. The researchers characterize the performance of the polyclonal Aflatoxin B1 antibody using serial dilution of homogenized tissue. They coat 96-well plates with the polyclonal antibody (the capture antibody), add the homogenized tissues with different dilutions of Aflatoxin B1, add the secondary antibody conjugated to horseradish peroxidase (the detection antibody), add the substrate for horseradish peroxidase, and analyze the reactivity and sensitivity using an ELISA reader. The researchers use purified Aflatoxin B to define a standard curve for testing assay sensitivity and toxin levels.

Specific Aim 2: The researchers obtain fresh tissue samples from chickens sold at local markets. To achieve this Aim, the researchers purchase live chickens from live poultry markets throughout their city. The researchers transport the live chickens to their university laboratory, where they euthanize them, drain and collect a sample of the blood, and conduct a necropsy of all other tissues. Each sample is labeled before being processed, and stored at either 4ºC or -20ºC.

Specific Aim 3: The researchers detect and quantify Aflatoxin B1 levels in tissues obtained from the chickens purchased at the live poultry market. To address this Aim, the researchers homogenize and prepare the tissue samples according to their newly-developed ELISA method. They seed 96-well plates with their samples and with positive and negative controls, and proceed to follow the ELISA method they developed in Specific Aim 1. They use an ELISA reader to detect and quantify the levels of Aflatoxin B1 in their samples.

Notional Research Project 3

Project Title: Elucidating the Mechanisms for Resistance of *Mycobacterium tuberculosis* to Rifampicin

Brief Summary: The treatment of *Mycobacterium tuberculosis* infection relies on the administration of a combination of antimicrobial agents, one of which is rifampicin, a bacterial RNA polymerase inhibitor. Resistance to this agent is a rapidly evolving concern; hence, elucidating the mechanisms by which resistance arises and counteracting them has been the focus of multiple research groups. One such group at University X has submitted a research proposal for approval that includes incubating a rifampicin-susceptible *M. tuberculosis* strain with successive increasing concentrations of rifampicin to select for rifampicin-resistant bacteria that arise in culture. The researchers will culture the resistant isolates in various concentrations of rifampicin and their protein, DNA, and RNA will be subsequently extracted. Protein and RNA will be used in expressional analysis studies whereas the DNA will be shipped to a collaborator abroad for sequence analysis. The researchers also will send the resistant *M. tuberculosis* isolates to this collaborator for possible further analysis, including metabolomic studies. The research team proposes to identify novel mutations and mechanisms that result in resistance to rifampicin. The virulence potential of the resistant isolates also will be analyzed *in vivo* using a mouse model of tuberculosis. Resistant isolates also will be examined for their susceptibility to various combinations of antimycobacterial agents *in vitro*. Treatments that prove effective in culture then will be assessed for their possible therapeutic potential by investigating their efficacy in mice infected with the rifampicin-resistant isolates.

Project Goal: The goal of these studies is to identify the cause of antibiotic resistance of *M. tuberculosis*, which could enable the development of new antibiotics effective against antibiotic-resistant *M. tuberculosis*, detection of antibiotic resistant strains in infected individuals, and/or future evaluation of treatment strategies that minimize inadvertent development of antibiotic resistance.

Specific Aim 1: The researchers seek to create rifampicin-resistant variants of M. tuberculosis. To achieve this Aim, the researchers incubate a laboratory strain of *M. tuberculosis* with rifampicin. The researchers conduct successive incubations, each with increasing concentrations of rifampicin. After the final incubation, the researchers collect the rifampicin-resistant bacteria, storing some bacteria in the -20ºC freezer and preparing some bacteria for molecular analysis.

Specific Aim 2: The researchers examine bacterial gene expression in the rifampicin-resistant bacteria. To address this Aim, the researchers grow the resistant bacteria in media containing different concentrations of rifampicin. After overnight growth, the researchers collect the bacteria and divide each pellet into four samples. The researchers extract protein from one sample, mRNA from the second sample, and genomic DNA from the third sample, and store the fourth sample. The researchers analyze gene expression by examining protein size and levels using Western Blot assays and mRNA size and levels using Northern Blot assays.

Specific Aim 3: The researchers analyze genomic DNA of rifampicin-resistant bacteria. The researchers send the isolated DNA (Specific Aim 2) and the bacterial samples to collaborators who have the ability to sequence and analyze the DNA, and acquire and analyze metabolomic data.

Specific Aim 4: The researchers examine whether the rifampicin-resistant bacteria are susceptible to other common antimycobacterial agents such a isoniazid and ethambutol. To achieve this Aim, the researchers tests antibiotic susceptibility of the rifampicin-resistant bacteria using *in vitro* disc assays. The researchers conduct *in vivo* therapeutic studies by infecting mice with the rifampicin-resistant *M. tuberculosis* followed by administering of effective antibiotics.