United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

USNPRC Institutional Biosafety Committee Meeting Minutes for review of registrations involving recombinant or synthetic nucleic acid molecules, Tuesday, September 23, 2025, 1:00PM-3:30PM

This meeting was held in USNPRC Building 45, Room 205.

Meeting attendees: Nick Chaplinski, Nancy Mead, Erica Spackman, Tim Satterlee, Doug Waltman, Darrell Kapczynski, Marcus Head, Jeff Scarbrough, Taejoong Kim

Not present: Tony Glenn, Jonathan Frye (excused)

Committee Chair: Erica Spackman
Biosafety Officer: Nick Chaplinski

Meeting Call to Order 1:45PM

New Business

2023-09, Tony Glenn, *Recombinant DNA Research Involving Mycotoxin Producing Fungi* (new submission)

- **Name of agents:**
  The primary fungi of interest to TMRU includes the mycotoxin producing species Fusarium verticillioides (Fv), Fusarium graminearum (Fg) and Aspergillus flavus (Af). They produce FUM, DON, and AFLA toxins, respectively. *Agrobacterium tumefaciens* for transformation.
- **Objectives:**
  The Toxicology & Mycotoxin Research Unit (TMRU) focuses on the food and feed safety concerns arising from mycotoxin-producing fungi. These fungi, primarily in the genera Fusarium and Aspergillus, are of significant concern due to their ubiquitous presence in major agricultural crops, such as corn, and their potential to produce very harmful mycotoxins causing various health and developmental concerns in animals and humans (Bennett and Klich, 2003; Glenn et al., 2007; Bolton et al., 2016; Blacutt et al., 2018).

  A separate registration submission (2022-21 Glenn) addresses the biosafety aspects of the mycotoxins and the producing fungi that we focus on as part of our mission. To address the recombinant DNA aspect of our research, we submit this registration. We routinely create gene deletion mutants and add-back complemented strains to functionally characterize the traits and features conferred by genes of interest.
- **Training**:
  Complete
- **NIH designation**:
  III-D-1-a
- **Biosafety Levels**:
  BSL-1, BSL-2

IBC Discussion:
The committee discussed the work described in the registration, especially how the constructs are characterized and what would be the procedure to follow if one is found to have an unexpected phenotype.

The committee requested more clarifications and revisions to this registration. The committee requested that the PI address the procedure for unexpected phenotypes within the document. Other revisions were

administrative.
A motion was proposed to make the necessary revisions and approve the updated registration administratively.

1st Kapczynski
2nd Mead

2025-05, Steven Conrad, *Bacterial surface display for poultry vaccination* (new submission)

- **Name of agents:** *E. coli* nissle 1917 strain
- **Objectives:**

There are many approaches to vaccination, but most of them can be thought of as the process of providing proteins from disease-causing microorganisms to an animal in a harmless context (i.e., such that they do not cause overt disease). These proteins (antigens) allow the immune system of the animal to create antibodies against the vaccine antigen, making the subsequent immune response to the analogous disease-causing organism faster and stronger upon exposure of the vaccinated animal to the disease-causing organism. A recent and ingenious approach to vaccination involves the use of bacteria to synthesize and display antigens from disease-causing microorganisms on their surfaces (this is referred to as "bacterial antigen surface display." These surface-displayed antigens are fed to an animal (usually by the oral route) and, upon contact with the gastrointestinal tract, can elicit a strong mucosal and systemic immune response. This approach offers several advantages. Bacteria are easy to genetically engineer, and the plasmids used in bacterial genetic engineering are simple to modify using basic cloning techniques. This IBC is intended to cover the insertion of different important protein antigens and/or marker proteins (such as fluorescent proteins) into bacterial expression plasmids for use in bacterial surface display vaccination. Each protein intended for a plasmid construct will be detailed in a separate amendment to this IBC.

- **Training:**
  Complete
- **NIH designation**:
  Section III-D-4-a
- **Biosafety Levels:**
  (A)BSL-2E

IBC Discussion:
The committee discussed the work described in the registration, focusing on how strains are chosen and the possibility of plasmids being able to jump into another bacterial species once in the environment.

The committee requested more clarifications and revisions to this registration. The committee requested that the PI provide an explanation for choosing strains and submitting a risk assessment on whether chicken feces and waste need to be treated differently than current protocol. Other revisions were administrative.

A motion was proposed to make the necessary revisions and approve the updated registration administratively.

1st Spackman
2nd Satterlee

2025-07, Stephen White, *Testing influence of envelope cholesterol in influenza diagnostic assays* (new submission)

- **Name of agents:** Influenza virus A/gyrfalcon/Washington/41088-6/2014
- **Objectives:**

Most analyses assume that influenza virus is either active (infective) or not. However, there is a mechanism where influenza virus can be reversibly inactivated by depletion of cholesterol. Cholesterol is necessary for membranes to bend extensively, and it is required for the influenza

virion envelope to bend and fuse with the cell membrane for initial infection. Depletion of cholesterol by 1 log was shown to dramatically reduce influenza virion infectivity, and addition of cholesterol to culture media could restore the infectivity of the influenza virus.

Since a standard assay for detecting influenza virus in eggs does not provide cholesterol, it is possible that this assay might miss influenza virions that could be restored to infectivity by cholesterol. We propose to test an alternative protocol that leverages egg yolk (where cholesterol is abundant) to see if it performs better for detecting influenza in general and especially for fermented food products where cholesterol depletion can occur naturally.

- **Training:**
  Complete
- **NIH designation**:
  Section III-E
- **Biosafety Levels:**
  BSL-2E

IBC Discussion:
The committee discussed the work described in the registration. The committee recommended that the rDNA section did not need to be checked on the form since the PI is using a reverse genetics made LPAIV prepared by another researcher and approved by the IBC

Other revisions requested by the committee were administrative.

A motion was proposed to make the necessary revisions and return the revised registration for DMR review and approval (Chaplinski, Kapczynski, Mead).

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1st Spackman
2nd Kapczynski

2025-08, Stephen Spatz, *Infectious laryngotracheitis virus-based recombinants expressing antigens of avian influenza A and immunoglobulins* (new submission)

- **Name of agents:** Infectious laryngotracheitis virus (CEO strain)
- **Objectives:**
  Our project aims to create a new, effective vaccine against highly pathogenic avian influenza (HPAI) by genetically engineering a poultry virus. We plan to use the infectious laryngotracheitis virus (ILTV), which is already used as a vaccine in chickens, as a "delivery vehicle." Our goal is to make this virus carry and express two key components: protective antigens from the HPAI virus and a therapeutic antibody. This two-pronged approach is designed to provide broader and more robust immunity than existing vaccines. We selected our antigens using a computational approach that analyzed hundreds of H5N1 influenza virus sequences currently circulating in the US.
- **Training:**
  Complete
- **NIH designation**:
  Sections III-D-1-a and III-D-2-a
- **Biosafety Levels:**
  BSL-2E

IBC Discussion:
The committee discussed the work described in the registration, esp. would the recombinant Influenza A/ILTV be more pathogenic than the parental vaccine. The committee recommended revisions to the document that were administrative in nature.

A motion was proposed to make the necessary revisions and approve the updated registration administratively.

1st Spackman
2nd Kim

2025-09, Stephen White, V*alidating loss of function to identify molecules supporting host-pathogen interaction* (new submission)

- **Name of agents:** Salmonella enterica Enteriditis, Campylobacter jejuni B540.
- **Objectives:**
  Salmonella hides from the host immune system by invading host cells (particularly macrophages) where the antibody response cannot reach. Preliminary data exists from human cell culture identifying host genes and pathways important for Salmonella cellular invasion and intracellular replication (Yeung et al 2019). We will use the human cells as models and test how similar Salmonella behaves in avian cells compared to human cells. Specifically, we will use precision gene editing techniques in avian cell culture to test loss-of-function for key genes. Our goal will be to achieve validation of molecules/genes required for host-pathogen interaction. These experiments will 1) empower understanding of how Salmonella invades host cells and 2) provide ways to address and possibly block cellular invasion. The simplest example would be through use of key genes identified from cell culture applied in traditional breeding of chickens (no gene editing). Such chickens would be selectively bred to be less susceptible to Salmonella.
- **Training:**
  Complete
- **NIH designation**:
  Section III-D-8
- **Biosafety Levels:**
  BSL-2

IBC Discussion:
The committee discussed the work described in the registration and recommended revisions to the document that were administrative in nature.

Motion for the revised registration to be approved administratively.

1st Spackman
2nd Kapczynski

Meeting adjourned: 4:05PM