

INSTITUTIONAL BIOSAFETY COMMITTEE MEETING

April 5, 2023
Zoom Meeting

MEMBERS PRESENT: *Chair:* Elizabeth Fozo, *Vice Chair:* Deidra Mountain, Feng Chen, Lori Cole, Paul Dalhaimer, Lezlee Dice, Doris D'Souza, George Dizikes, Brittany Isabell, Jun Lin, Joseph Jackson, Jae Park, Jessica Vélez

Ex-Officio: Bryan Cranmore, Carolina Dolislager, Linda Hamilton, Brian Ranger, Daniel Thomas, Jessica Woofert

MEMBERS ABSENT: Marc Caldwell, Reza Hajimorad, Ling Zhao

OTHERS PRESENT: David Anderson, Cassie Halvorsen, Sreekumari Rajeev, Qixin Zhong

Opening:

The IBC Vice Chair called the meeting to order at 10:00 AM. The minutes for March 1, 2023, IBC Meeting were reviewed and approved as written.

Full Member Review IBC Registrations:

#IBC-06-288-1 (Juan Luis Jurat-Fuentes) Recombinant DNA Registration, III-E-1, Amendment

Dr. Jurat-Fuentes' research covers the study of the expression of various insect gut epithelial proteins in 1) common lab strains of *Escherichia coli* for purposes of purification and antisera development, 2) various insect cell lines to assess their ability to act as functional receptors for the Cry toxins of *Bacillus thuringiensis* and 3) characterizing insecticidal mode of action. The committee voted to approve the registration pending correction of autoclave validation dates, updating the waste contractor information, and clarification of dsRNA containment measures. The amendment includes an addition to personnel, grant information, biosafety cabinet information, insect host cell lines, *B. thuringiensis* vectors and promoters, B2 protein of flock house virus (FHV)-linker-Yellow fluorescent protein N terminus, VP35 protein of Marburg virus (MARV)-linker-Yellow fluorescent protein C terminus, and the addition of a dsRNA binding-dependent fluorescence complementation (dRBFC) assay to monitor dsRNA distribution and dynamics in vivo is being constructed. The containment was set at BSL-1. The committee approved pending the correction of typographical errors in the Nontechnical and Technical summaries.

#IBC-14-416-2 (Qixin Zhong) Infectious Agents and Nanoparticles, 3rd-year Rewrite

Dr. Zhong was present to discuss their research involving determining the minimum inhibitory and minimum bactericidal concentrations of natural antimicrobials (essential oils, lysozyme, nisin, etc.) necessary to inhibit or inactivate foodborne pathogens. The study will also cover the time-kill growth kinetics of pathogens in microbiological growth media and/or in food matrices such as fruit juices, milk, cheeses, and fresh produce containing antimicrobial compounds. Procedures involve growing small-scale cultures of *Escherichia coli*, *Salmonella enterica* (non-typhi), *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis*, exposure to natural antimicrobial compounds, and standard plating for enumeration. Containment and safety practices were set at BSL-2. The committee sent the registration to be approved through

Designated Member Review pending the correction of a typographical error in the Nontechnical Summary and clarification of whether waste will be autoclaved or bleached.

#IBC-14-420-1 (Neal Stewart) Recombinant DNA Registration, III-E-2-a, 3rd-year Rewrite

Cassie Halvorsen was present to discuss Dr. Stewart's research covering the development of gene activation and repression targeting biotechnology. Briefly, a transcriptional activator and repressor system derived from *Neurospora crassa* will be used to selectively turn on/off bioreporter (pporRFP) gene expression. The system will be tested on tobacco leaves (*Nicotiana tabacum*). Standard Agrobacterium-based plant transformation techniques will be used. Containment was set at BSL-1/BL- 1-P. The committee sent the registration to be approved through Designated Member Review pending corrections that include a rewrite of the Nontechnical Summary to include the purpose, research design, project procedures, and outcome measures; clarification if Ecdysone is or is not inducible and is listed correctly; and the correction of "7:10" to read 70% for Ethyl / Isopropyl Alcohol in the disinfectant table.

#IBC-20-544-2 (David Anderson) Infectious Agents, 3rd-year Rewrite

Dr. Anderson was present to discuss his registration covering the use of *Staphylococcus aureus* strains in his DO-PRORP-funded grant studying functional bone regeneration in a cortical defect in goats with and without infection. Containment was set at ABSL-2. The committee approved the registration pending the clarification of the PRORP abbreviation in the project title, the removal of Dr. Kania, the addition of Dr. Kania's replacement, clarification of how animals are euthanized, and the carcasses are transported, and the indication of ABSL-2 risk level for the JRTU location.

#IBC-20-545-2 (David Anderson) Infectious Agents, 3rd-year Rewrite

Dr. Anderson was present to discuss his study of a hydrophilic bone tissue regeneration scaffold which can serve a dual role as a local antibiotic delivery device to eliminate bacteria from contaminated orthopedic sites in a goat model. Containment was set at ABSL-2. The committee approved the registration pending the correction of a typographical error in the Nontechnical Summary; the addition of IACUC protocol number 2776; removal of references to Dr. Kania; the addition of Dr. Kania's replacement; the correction of the VREC location to JRTU and an indication that the risk level is ABSL-2; an update to the biosafety cabinet certification dates; and the removal of "N/A" for Question 8.6 in the Facilities and Procedures Assessment Section.

#IBC-23-587-2 (Sreekumari Rajeev) Infectious Agents, Nanoparticles, and Recombinant DNA, III-D-2-a, New Registration

Dr. Rajeev was present to discuss their research on vaccine-challenge studies of clinical Leptospirosis infection and protection using hamster and mice models. Their goal is to develop a vaccine that can protect from immediate and and-long term infection. They will use the mice model to preliminary evaluate the vaccine candidates and then move selected candidates for testing in the hamster model. Containment was set at ABSL-2. The committee sent the registration to be approved through Designated Member Review pending an update to personnel; clarification under project procedures that recombinant vaccines are formed by conjugating the peptides to the polymeric nanoparticles; separation of each agent into individual line items for the Infectious Agents section; the addition of IACUC protocol numbers 2968 and 2975; the addition of a statement in the Technical Summary about the preparation of nanoparticles, more detail about the preparation of vectors and growing of *E. coli*, and the removal of Mossman 120D under the animal procedures that will be performed; an update to the biosafety cabinet certification dates; removal of the refrigerator listed for Mossman; and indicating that disposable PPE will be work when working hamsters and mice.

#IBC-23-588-2 (Mohamed Abouelkhair) Recombinant DNA, III-D-1-a, New Registration

Dr. Abouelkhair's research covers the characterizing and comparing cfDNA from dogs with B-cell lymphoma to cfDNA from healthy dogs to investigate the potential of plasma cell-free DNA (cfDNA) as a non-invasive diagnostic and monitoring tool for canine B-cell lymphoma. Containment was set at BSL-2. The committee sent the registration to be approved for Designated Member Review pending the confirmation that no other personnel will be needed; clarification in the Nontechnical Summary about how the CRISPR will be used, what is targeted, and if the Cas component is being purified; the completion of the Human Derived Materials section; update the Recombinant DNA (rDNA) Section to include the K562 and ChoK1 cells as well as any relevant vectors in the rDNA Section; update the Recombinant DNA (rDNA) Section to include Lenti LUCOS plasmids; clarification in the Technical Summary as to the purpose of the CRISPR system, what genes will be targeted, how the system is implemented, the purpose of the flow cytometry assays, and confirmation that lentiviruses are not being produced and that the PI is using these plasmids for their CRE expression; correction of bleach contact times to be consistent throughout the registration; and completing the Animal Carcasses/Pathological Waste section.

#IBC-23-589-2 (Cong Trinh) Infectious Agents and Recombinant DNA, III-D-1-a, New Registration

Dr. Trinh's research covers the study of *Candida auris* and its rapid emergence as a multidrug-resistant yeast that can cause severe systemic infections in immunocompromised individuals. In recent years, *Candida auris* infections have quickly spread across the globe, and since the COVID-19 pandemic, they have become increasingly widespread in hospitals across the United States. Due to the rapid spread and innate drug resistance of *Candida auris*, there is a need to study this pathogen at the genomic level such that new drugs and prevention measures may be developed and implemented. CRISPR-Cas technology can precisely remove or insert genes to elucidate their function. This project aims to utilize CRISPR-Cas technology to systematically knockout genes across the genome in either a single gene or multiplex fashion and subject these strains to antifungal drug treatments (e.g., azoles, echinocandins, and polyenes) and biofilm formation assays. From these screens, they can understand what genes are related to conditions that may cause resistance or infection and what genes are essential for the pathogen's survival. Containment was set at BSL-2. The committee approved the registration pending the clarification of "various conditions" in the Nontechnical Summary; clarification of what is meant by "low concentrations ($<10^8$ - 10^9 cells/mL)," and the volume being generated; the addition of a statement indicating that dedicated lab coats or disposable coats are used for *Candida auris* work; the correction of "7:10" to read 70% for Ethyl / Isopropyl Alcohol in the disinfectant table; indicating that *Candida auris*-containing waste is autoclaved before disposal into Advantra bins; clarification that lab-specific training will be needed; and correction of minor typographical errors.

Old Business:

Administrative Report

i. Contingencies

Following up on the March 1, 2023, meeting, Dr. Abouelkhair's tabled registration (#16-447-2) was terminated. Dr. Chunlei Su's registration (#05-232-2) was approved through Designated Member Review to include the removal of the vaccine development statement, clarification about the type of environmental samples used, clarification about serology testing, the addition of the biosafety cabinet certification date, clarification about lab coating laundering and autoclaving, the addition of goggle disinfection, the correction of the bleach disinfection time from 5 to 10 minutes, and clarification about the use of unpowdered latex or

nitrile gloves. Dr. Dallas Donohoe's registration (#13-410-2) was still pending response and the correction of typographical errors in the nontechnical summary; the addition of language in the technical summary indicating where the mice are obtained from and that they are for breeding purposes and that no rDNA work will be done; an update to the IACUC protocol number; the correction of the bleach contact time to 10 minutes; clarification of how liquid waste is disposed of; and unchecking the autoclaves listed for Mossman and checking medical contractor (Advantra) in the solid/non-sharp waste section. Dr. Alessandro Occhialini's registration (#23-586-2) was approved through Designated Member Review to include the addition of a brief protocol for pollen collection and RNAseq in the technical summary; the addition of more detail regarding soil work; the addition of genes of interest for the proposed work for photosynthesis, defense against pathogens, and biofortification; clarification about how *Nicotiana benthamiana*, *Arabidopsis thaliana*, and *Physcomitra patens* will be used; clarification about what "heterologous pathway" means; correction of "13 GOI" to read "14 GOI" in the technical summary; and the removal of unnecessary details for Golden-gate cloning.

ii. *Administrative Approvals*

The Biosafety Officer administratively approved Dr. Brad Binder's amendment to registration (#08-331-1) on 3/24/2023 for updates to personnel and grant information, the addition of Hesler Room 103, the removal of Hesler 105, the addition of an -80°C freezer, incubator, and refrigerator, and an update to the spill response, including disinfectants and contact times. The Biosafety Officer administratively approved Dr. Neal Stewart's amendment to registration (#12-379-1) on 3/23/2023 for updates to personnel, the addition of Switchgrass (*Panicum virgatum*), clarification that *Pseudomonas putida* will be used to launch stimuli-sensed signals to plants, an update to the biosafety cabinet certification date, and an update to the autoclave validation date for solid/non-sharp waste. The Biosafety Officer administratively approved Dr. Girish Neelakanta's amendment to registration (#21-560-2) on 3/23/2023 to add *A. phagocytophilum*-infected *Haemaphysalis longicornis* in the in vitro laboratory conditions to the Technical Summary. No animal work will be performed with these infected ticks. The Biosafety Officer administratively approved Dr. Joseph Jackson's amendment to registration (#21-573-2) on 3/23/2023 for updates to personnel, grant information, IACUC protocol number, biosafety cabinet certification dates, bleach contact time, spill response, shipping activities, and autoclave validation date. The Biosafety Officer administratively approved Dr. Hameeda Sultana's amendment to registration (#22-579-2) on 3/23/2023 for updates to the biosafety cabinet information and certification date, the addition of a clarification that while autoclaving of research materials may occur in LAF and/or UTCVM autoclaves, the ultimate disposition is in the cage where Advantra picks up waste, and the medical contractor is indicated as Advantra in the solid/non-sharp waste section.

iii. *Administrative Terminations*

Dr. Mohamed Abouelkhair's registration was terminated on 3/23/2023.

iv. *Administrative Exemptions:*

None.

v. *Accidents, Injuries/Exposures:*

Brian notified the committee that there was one recent injury at the Forensic Anthropology

Center. The student scratched their finger with a dental instrument used to extract mummified tissue. Linda is scheduled to perform a follow-up with the department.

vi. Laboratory Report:

Linda notified the committee that LSS is performing preliminary audits and meetings with department heads and laboratory safety advocates in March.

vii. iMedRIS Update, Manual Reviews, & System Orientation:

None.

IBC Registration Form (Section 6)

Jessica notified the committee that this form section would be tabled for the next meeting as additional time is needed to evaluate and draft the recombinant DNA section of the IBC form.

IBC Registration Form (Section 7)

Jessica asked the committee to review the Infectious Agents section of the IBC registration form and to send comments before the next meeting.

New Business:

Safety Stratus

Jessica notified the committee that the Biosafety module for Safety Stratus had been approved and is scheduled for integration after the Chemical Safety module is completed.

The meeting adjourned at 12:06 AM. The next meeting is scheduled for May 3, 2023, from 10:00 am to 12:00 pm EST at the Institute for Advanced Manufacturing Materials (IAMM) Building, Room 145, via Zoom.